

Chronic versus acute ingestion of sodium citrate: a randomized placebo controlled cross-over trial for swimming 200 metres in well-trained swimmers age 13-17

by

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Abstract

A double-blinded, placebo controlled, cross-over design was used to investigate sodium citrate dihydrate (Na-CIT) supplementation improve 200m swimming performance. Ten well-trained, male swimmers (14.9 ± 0.4 y; 63.5 ± 4 kg) performed four 200m time trials: acute (ACU) supplementation (0.5g/kg), acute placebo (PLC-A), chronic (CHR) (0.1g/kg for 3 days and 0.3g/kg on the 4th day pre-trial), and chronic placebo (PLC-C). Na-CIT was administered 120min pre-trial in solution with 500mL of flavored water; placebo was flavored water. Blood lactate, base excess (BE), bicarbonate, pH, and PCO_2 were analyzed at basal, 100min post-ingestion, and 3min post-trial via finger prick. Time, lactate, and rate of perceived exertion were not different between trials. BE and bicarbonate were significantly higher for the ACU and CHR trials compared to placebo. “Responders” improved by 1.03% ($P=0.043$) and attained significantly higher post-trial lactate concentrations in the ACU versus PLC-A trials and compared to non-responders in the ACU and CHR trials.

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List of Abbreviations

ACU	Acute
ALD	Aldosterone
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BE	Base excess
CHR	Chronic
CIT	Citrate anion
Cl ⁻	Chloride ion
DA	Dopamine
EPI	Epinephrine
GI	Gastrointestinal
H	Hypoxia
H ⁺	Hydrogen ion
HH	Hypobaric hypoxia
h-CHO	High carbohydrate
K ⁺	Potassium ion
MVC	Maximal voluntary contraction
Na ⁺	Sodium ion
NaCl	Sodium chloride
Na-CIT	Sodium citrate
NaHCO ₃	Sodium bicarbonate
NaLac	Sodium lactate
NE	Norepinephrine
NH ₄ Cl	Ammonium chloride
No	Normoxia
PaCO ₂	Arterial partial pressure of carbon dioxide
PaO ₂	Arterial partial pressure of oxygen
PCO ₂	Venous partial pressure of carbon dioxide
PFK	Phosphofructokinase
PLC	Placebo
PLC-A	Acute placebo
PLC-C	Chronic placebo
RER	Respiratory exchange ratio
RPE	Rate of perceived exertion
VE	Ventilation rate

Chapter 1: Introduction

1.1 Rational

Anaerobic glycolysis quickly provides adenosine triphosphate (ATP) for muscle contraction during high intensity, short duration exercise. The fast rate of glycolysis during anaerobic exercise results in pyruvate formation exceeding pyruvate oxidation and results in a buildup of lactic acid (Wells et al. 2009, Street et al. 2005). Lactic acid dissociates quickly to lactate and hydrogen ions (H^+), which causes a decrease in muscle and blood pH (Wells et al. 2009). The increase in H^+ causes impaired release of calcium from the sarcoplasmic reticulum and calcium ion binding that inhibits the coupling of actin and myosin (Katz et al. 1984, Requena et al. 2005). This intracellular acidosis is directly related to fatigue that is defined as a decrease in force production with an increased perception of effort (Enoka & Stuart 1992).

Ingestion of sodium citrate (Na-CIT), an alkalizing agent, increases extracellular pH as it is metabolized to bicarbonate. Studies have confirmed (Katz et al. 1984, McNaughton 1990, McNaughton & Cedaro 1992, Oopik et al. 2003, Robergs et al. 2005) that increasing extracellular pH facilitates the efflux of Lactate and H^+ from active muscles compared to placebo. Therefore, artificially inducing alkalosis prior to anaerobic exercise may reduce intracellular acidosis and increase the time to fatigue.

Researchers have studied sodium bicarbonate (Katz et al. 1984, McNaughton et al. 2008) and Na-CIT (Jain et al. 2003, Kowalchuk et al. 1989, Linossier et al. 1997,

McNaughton 1990) as potential alkalizing agents. A meta-analysis by Carr et al. (2011) reported that ingestion of sodium bicarbonate at a dose of 0.3g/kg, 90min pre-trial enhances anaerobic performance, but the ingestion of Na-CIT did not appear to alter anaerobic performance. Indeed, Na-CIT has been studied over a broad array of doses, times, and events with inconclusive results (Linossier et al. 1997, McNaughton 1990, McNaughton & Cedaro 1992, Oopik et al. 2003, Oopik et al. 2004, Potteiger et al. 1996, Zochowski et al. 2009).

McNaughton et al. (1990, 1992) investigated the Na-CIT for the optimal dose and performance duration to elicit a potential ergogenic effect. They concluded that 0.5g/kg of body mass, 90 - 120min prior to maximal effort represented the optimal conditions for a potential ergogenic effect in events lasting 60 - 240s. In the case where Na-CIT was given at a dose of 0.6g/kg, 5 of the 8 participants developed gastrointestinal (GI) discomfort which negatively impacted performance (Schabort et al. 2000). Nonetheless, Na-Cit exhibited an improved GI acceptance profile over the more commonly used sodium bicarbonate (Requena et al. 2005).

Na-Cit is not banned by the world anti-doping agency (WADA 2013 Prohibited List); however, it is not as popular as a supplement compared to sodium bicarbonate due to the inconsistent performance effects found in the literature. On the other hand, Na-Cit has been suggested to have less GI discomfort than sodium bicarbonate and, therefore, may be a superior alternative. A randomized, cross-over, placebo controlled trial, as is proposed in this study, would be the best method to prove the potential benefit of Na-Cit on swimming performance in an anaerobic event. Furthermore, testing a novel

chronic dosing regimen may eliminate any GI symptoms that hinder performance when ingesting alkalizing agents at high, acute doses. Lastly, this will also be the first study that looks at the highly competitive adolescent population in swimming. The competitive nature of sport has presented an atmosphere where 80% of high level adolescent athletes are using supplements and other non-doping strategies to improve performance without any research into the effectiveness of such ergogenic aids in the young population (Braun et al. 2009).

1.2 Objectives and Hypotheses

1.2.1 Research Objectives

The main objective of this study was to evaluate the impact of multiple dosing regimens of sodium citrate ingestion on exercise performance, hematological variables, and parameters of aerobic and anaerobic physiology in well-trained adolescent male swimmers. Specifically, a double-blinded, placebo controlled, cross-over design was used to investigate whether Na-CIT ingestion improves 200m swimming performance in male adolescent swimmers. Differences between acute versus chronic Na-CIT supplementation will also be examined.

The primary outcome variable was performance time (s) and the secondary variables was lactate concentration (mmol/L), rate of perceived exertion (RPE), venous

partial pressure (PCO₂) (mmHg), base excess (mmol/L), and bicarbonate concentration (mmol/L).

1.2.2 Research Hypotheses

The null hypotheses that were evaluated were: i) sodium citrate ingestion (chronic and acute) will not have an effect on exercise performance compared to placebo; and ii) there will be no difference between chronic and acute treatments on the 200m performance times.

Chapter 2: Review of Literature

2.1 Bioenergetics of High-Intensity Exercise

Three energy systems are utilized for ATP turnover and resynthesis in the human body: 1) the alacti anaerobic system, 2) the lactic anaerobic system, and 3) the aerobic oxidative system. At the onset of exercise all energy systems contribute to energy production but their relative contribution varies as a function of the duration and intensity of the exercise (Wilmore & Costill 2004). The alactic anaerobic system derives its energy from the breakdown of creatine phosphate and is the primary source of energy provision for 12-15 seconds. The lactic anaerobic system takes over as the principal energy supplier through the breakdown of muscle glycogen to lactate for the production of ATP (anaerobic glycolysis). The body relies on anaerobic glycolysis as the primary energy system for high-intensity, short duration exercise lasting greater than 12 – 15 seconds and less than 2min (Wilmore & Costill 2004). Quantification of anaerobic energy release and, hence, its percent contribution to energy production for a given amount of time can be analyzed directly by measuring lactate production and phosphocreatine breakdown or indirectly by accumulated oxygen deficit (Medbo & Tabata 1989). When exactly during high-intensity exercise the anaerobic energy provision reaches 50% with aerobic oxidative metabolism contributing the other 50% is a source of contention and may stem from how anaerobic energy release is quantified and the muscle mass engaged in a given exercise (Medbo & Tabata 1989, Spriet et al.

1987). None-the-less, at 60 - 120s the aerobic oxidative system takes over as the primary source of energy provision. This is accomplished by the breakdown on muscle glycogen, in the presence of oxygen, for a more efficient production of ATP. The pyruvate derived from the breakdown of glucose then converted to Acetyl-CoA as a substrate for the Krebs cycle. Furthermore, the breakdown of free fatty acids plays an important role in the aerobic energy production (Wilmore & Costill 2004).

2.2 Anaerobic Metabolism

Energy production via anaerobic glycolysis takes place in the cytoplasm of each skeletal muscle cell by catabolism of glucose or muscle glycogen to pyruvate through 10 enzyme driven steps (Spriet et al. 2000). The ATP derived from glycolysis is rendered at a high rate for rapid and powerful muscular contraction; however, with such high rates of glycolytic flux, pyruvate formation exceeds pyruvate oxidation. Oxidized pyruvate is converted to lactic acid to maintain the rate of anaerobic glycolysis and energy production (Wells et al. 2009). Athletes performing sporting events, such as swimming, downhill skiing, 800m run, 1500m speed skating, and most team sports, rely on anaerobic metabolism as the primary driver for energy provision.

Lactic acid dissociates quickly to lactate and H^+ which causes a decrease in muscle and blood pH (Costill et al. 1984). In order to maintain exercise at high intensity, removal of lactic acid from Type II glycolytic muscle fibres, where lactate is predominantly produced, to Type I oxidative fibres, where lactic acid can be oxidized in

mitochondria, must be efficient. The transfer of lactate and H^+ is accomplished via pH sensitive monocarboxylate lactate transporters located on the surface of the sarcolemma (Wells et al. 2009).

The lactate that quickly accumulates during high intensity exercise is a commonly measured variable in sport physiology. Typical values range from 1 - 2mmol/L at rest, to 4mmol/L in moderate sustainable exercise, to greater than 16mmol/L in maximal anaerobic exercise. There is a close relationship between blood lactate concentration and fatigue during high intensity exercise. However, this does not account for fatigue and cramps observed during high-intensity exercise in individuals with glycolytic/glycogenolytic disorders, in which glycolysis, and therefore lactate production, is blocked (Wells et al. 2009). Hence, many other variables may contribute to fatigue found in muscle cells. Indeed, the decrease in muscle and blood pH that corresponds with an increase in lactate is a major factor that produces fatigue (Noakes 2000). It is due to these factors, as well as the relative ease with which blood lactate can be measured, that lactic acid and lactate have been of considerable interest to exercise scientists for many years (Juel 1997, Stallknecht et al. 1998).

2.3 Fatigue

Fatigue is defined as a decrease in force production in the presence of increased perception of effort (Enoka & Stuart 1992). Since fatigue is a limitation to high-intensity exercise performance, minimizing fatigue and / or delaying the onset of fatigue is

desirable during competition. When energy needs are met primarily through anaerobic metabolism, several processes occur which lead to fatigue. The increase in H^+ concentration causes: i) an impaired release of calcium from the sarcoplasmic reticulum of the muscle cell, ii) an inhibition of the coupling between actin and myosin (Fitts 1994, Noakes 2000, Requena et al. 2005, Wells et al. 2009), iii) a decrease in the rate of glycolysis through an inhibition of a pH sensitive enzyme phosphofructokinase (PFK), the rate-limiting enzyme in the glycolytic pathway (Noakes 2000), iv) a reduction in ATP production (Fitts 1994), v) a reduction in the ATP utilization rate (Fitts 1994), and vi) a decrease in pH sensitive transporter activity, e.g., monocarboxylate lactate transporter (Wells et al. 2009), K_{ATP} transporter (Street et al. 2005).

Additionally, skeletal muscle releases potassium ion (K^+) during activity. K^+ accumulation reduces muscle excitability and is considered to be involved in both the development of muscle fatigue and blood flow regulation (Fitts 1994, Sostaric et al. 2006, Street et al. 2005). The capacity for K^+ re-uptake via the Na^+/K^+ pump and $Na^+/K^+/Cl^-$ co-transporter is exceeded during anaerobic exercise. Consequently, K^+ accumulates in the interstitium and plasma (Sostaric et al. 2006, Street et al. 2005).

2.4 Enhancement of Performance in Sport

Since fatigue is a limitation to high-intensity performance, its delay is desired during competitions. Ergogenic aids provide enhanced energy production, utilization, and recovery during competition in the form of a drug, natural health product, or other

intervention (Aherendt 2001). Products that claim to be performance enhancing are popular with recreational and elite athletes alike. However, due to the negative side effects of doping and banned substances, many athletes are now turning to ergogenic supplements as a safe and legal way to boost athletic performance.

Currently, athletes appear to be searching for legal alternatives; particularly nutritional ergogenic aids (Williams 1995). Nutritional ergogenic aids encompass a broad range of substances that include standard dietary constituents, like carbohydrates and proteins to atypical substances, such as sodium bicarbonate and creatine (Applegate 1999).

2.5 Alkalizing Agents

During anaerobic type maximal exercise (such as a 200-meter freestyle race), pH within the blood can decrease from 7.4 to 7.1, while muscle pH can decrease to as low as 6.8 (McNaughton et al. 2008). Muscle physiology has defense mechanisms that support the cell to keep pH in an acceptable range. Essentially this involves decreasing the concentration of intracellular free H^+ through intracellular mechanisms and extracellular buffering systems that favor the movement of H^+ between the muscle cell and the extracellular fluid. Throughout the body, strategies exist to offset the increased production of H^+ during maximal exercise such as i) acid excretion by the kidney (Oster et al. 1988), ii) hyperventilation during breathing (Wells et al. 2005), and iii) blood bicarbonate buffering (Requena et al. 2005).

The use of substances to buffer the production of H^+ during anaerobic type sporting competitions has gained in popularity for a number of years. Ingesting alkalizing agents has been suggested as a strategy to postpone the onset of fatigue during high intensity exercise by slowing the decline in muscle and blood pH (Katz et al. 1984, Kowalchuk et al. 1989). Studies have confirmed (Ibanez et al. 1995, Katz et al. 1984, McNaughton 1990, Robergs et al. 2005, Sostaric et al. 2006) that increasing extracellular pH, via an alkalizer, promotes the efflux of Lactate and H^+ from active muscles compared to placebo. Therefore, artificially inducing alkalosis prior to anaerobic exercise may reduce intracellular acidosis and increase the time to fatigue (McNaughton et al. 2008, Noakes 2000).

Currently, the process known as “bicarbonate loading” in which sodium bicarbonate is ingested pre-performance, is a popular method of blood alkalization with athletes. Numerous studies have examined the impact of sodium bicarbonate on performance in a broad array of modalities, distances, times, and doses. Indeed, a meta-analysis done by Carr et al. (2011) revealed sodium bicarbonate enhanced performance by 1.7% ($\pm 2.0\%$) for a 60s maximal effort, with a dose of 0.3g/kg of body mass being the optimal dose. However, the side-effects associated with ingesting the high doses needed to induce an adequate modification of the acid-base state, can be detrimental to performance (McNaughton et al. 2008, Requena et al. 2005, Schabert et al. 2000). The GI acceptance profile of sodium bicarbonate is narrow and few humans can adequately tolerate the doses needed to elicit an ergogenic effect (Oster et al. 1988).

Therefore, another alkalizing agent, Na-CIT, has increasingly been studied and is garnering interest from coaches and athletes.

2.5.1 Sodium Citrate

Na-CIT has been studied in many sports over a broad array of doses, times, and distances with inconclusive results (Table 1). Indeed, a meta-analysis by Carr et al. (2011) revealed no clear effect on performance. It is this uncertainty that has prevented Na-CIT from becoming a viable, and improved GI accepting, alternative to sodium bicarbonate.

Na-CIT is not found in body fluids. After ingestion, Na-Cit quickly dissociates into sodium (Na^+) and citrate (CIT) ions. The CIT anion, unlike the bicarbonate anion, is expelled from the plasma (Kowalchuk et al. 1989). Hence, the electrical equilibrium of cations and anions becomes unbalanced after ingestion. Neutrality is achieved by decreasing the concentration of H^+ and increasing bicarbonate concentration (Kowalchuk et al. 1989, Requena et al. 2005). Unlike sodium bicarbonate, this process is an indirect method of plasma alkalization, and is posited to be derived from liver oxidation (Requena et al. 2005).

The expelled CIT is capable of entering the sarcolemma (Linossier et al. 1997) through a recently discovered plasma membrane transporter - a plasma membrane citrate transporter (Sun et al. 2010). A comparison of sequence similarity, transport mechanisms, and types of inhibitors reveals that the plasma membrane citrate

transporter is distinctly different from the mitochondrial citrate transport protein (Sun et al 2010). Within the muscle cell, CIT is involved in several metabolic processes: i) it acts as a metabolic intermediary for the Krebs cycle (Wells et al. 2009), ii) it transports acetyl CoA from the mitochondrion to the cytosol for fatty acid synthesis (Wells et al. 2009), iii) it is a negative allosteric effector of phosphofructokinase (PFK) (Kemp & Foe 1983); however, this inhibitory effect may be exaggerated under normal physiological conditions (Peters & Spriet 1995); and iv) it can cause a reduction in the contraction threshold by means of its effect on the membrane's potential (Requena et al. 2005). Therefore, Na-CIT seems to have a greater effect on potential performance enhancement than just plasma alkalization and lactate/H⁺ kinetics. However, the nature of the mechanisms involved in the delay of exhaustion could be different and remains to be elucidated (Linossier et al. 1997).

2.6 Sodium Citrate Supplementation in Athletes

The published research investigating the ergogenic effect of sodium bicarbonate has reached the general conclusion that it aids in maximal intensity performance. However, Na-CIT is still in the experimentation phase of study, and at present, results point toward an unclear effect on performance. Though both substances have paralleled physiological behavior, the optimal protocol for each substance to elicit enhanced performance differs widely.

2.6.1 Finding the Optimal Protocol

McNaughton (1990) pioneered finding the optimal protocol to elicit an ergogenic effect with Na-CIT. He investigated whether administration of Na-CIT would enhance performance and if so, what would be the optimal dose to improve anaerobic performance in a 60s sprint. Using the doses 0.1 - 0.5g/kg of body mass in 0.1g/kg increments, 90min pre-trial, McNaughton tested the outcome variables of peak power and total work on a cycle ergometer. The minimal dose to bring about an improvement over control and placebo was 0.3g/kg with the optimal dose being 0.5g/kg ($P < 0.0001$). Although McNaughton (1990) tested up to 0.5g/kg, Schabert et al. (2000) investigated an acute dose of 0.6g/kg of Na-Cit and observed that GI discomfort negatively affected performance in 5 of the 8 subjects during a 40km cycling time trial. Following his experiment in 1990, McNaughton and Cedaro (1992) aimed to find the optimal durations of exercise performance to benefit from Na-Cit ingestion at 0.5g/kg, 90min pre-trial. The authors tested durations of 10, 30, 120, and 240s at maximal intensity on a cycle ergometer. Significant improvements in total work and peak power compared to placebo were found at 120 and 240s ($P < 0.05$) performance durations. Therefore, the authors concluded that ingestion of Na-CIT at 0.5g/kg 90min pre-trial would benefit maximal intensity exercise lasting 60-240s. However, unlike sodium bicarbonate, Na-CIT must be metabolized to bring about an increase in blood pH ~~and~~ bicarbonate concentration (Kowalchuk et al. 1989, Linossier et al. 1997). With this in mind, Potteiger et al. (1996) discovered that peak blood pH and bicarbonate concentration for Na-CIT

were reached at 120min post-ingestion compared to 90min for sodium bicarbonate. Though there is considerable inter-individual variability with respect to metabolization times (Potteiger et al. 1996, Schobart et al. 2000), the consensus is that Na-CIT ingestion should take place 90 – 120min pre-trial. In summary, administration of Na-Cit at a dose of 0.5g/kg, at 120min pre-trial, is the optimal protocol to elicit a potential ergogenic effect during maximal anaerobic performance.

2.6.2 Sodium Bicarbonate versus Sodium Citrate

Few studies have compared the effectiveness of sodium bicarbonate to that of Na-CIT using similar protocols (Parry-billings & Maclaren 1986, Potteiger et al. 1996, Tiriyaki & Atterbom 1995, Van Montfoort et al. 2004). Van Montfoort et al. (2004) compared sodium bicarbonate, Na-CIT, sodium lactate, and sodium chloride as a placebo in a run to exhaustion test. The test ran at a fixed speed that was derived during a familiarization session in order to elicit fatigue in 1-2min. Equimolar doses were ingested relative to body mass (3.6mOsmol/kg) 90min prior to the performance test. Even though blood bicarbonate values were highest for the CIT group, and were significantly increased over placebo, the sodium bicarbonate test revealed the greatest percentage of mean improvement at 2.7%, with 1.7% and 0.5% being reported for sodium lactate and Na-CIT, respectively. The authors reasoned that the high dose of 0.5g/kg of CIT may have inhibited ATP production through its effect on PFK compared to a common lower dose of 0.3g/kg. Unfortunately, the only data analysis done by Van Montfoort et al. consisted

of calculating percent mean improvement and percent chance of improvement when comparing the buffering agents (Table 1). Parry-Billings & MacLaren (1986) used doses of 0.3g/kg for both sodium bicarbonate and Na-CIT, as well as a combination of the two, and found no significant change in total work compared to placebo for 3 intermittent Wingate tests. However, the CIT trial did have the highest pH and bicarbonate concentration compared to sodium bicarbonate, which may have contributed to a higher mean and peak power in the second and third bouts. When comparing sodium bicarbonate (0.3g/kg) to Na-CIT (0.5g/kg) in terms of aerobic capacity, Potteiger et al. (1996) reported no difference between conditions. However, the authors suggested that it would be better to test athletes for a set distance or in a time trial than to use a set run velocity to exhaustion, as was the case here. The authors argued that based on the higher blood lactate in the CIT trial, if run velocity could change voluntarily, the greater efflux of H^+ from the cell due to the pH contrast could lead to an increased speed and greater power for the given amount of time.

2.6.3 Acute Effects during High-Intensity Sport Specific Exercise

McNaughton & Cedaro (1992) reported an ergogenic benefit of Na-Cit ingestion in cycling when recording total work and peak power output for durations of 60 - 240s ($P<0.05$) at a dose of 0.5g/kg. However, no ergogenic effect was observed for exercise durations of 10 - 30 seconds. Similarly, Linossier et al. (1997) reported time to exhaustion when cycling at 120% peak VO_2 was significantly extended. Interestingly, the

authors reported a large variability in response to CIT (1 - 44%), which was considered to be associated with the differences in the physical conditions of the subjects. Those with a lower peak VO_2 actually responded better than the more endurance trained subjects. Contrary to this observation, Shave et al. (2001) tested elite runners in a 3000m time trial, and reported a significant performance enhancement ($P<0.05$) during the CIT trial. Also in line with the study done by McNaughton & Cedaro (1992), Ibanez et al. (1995) tested six elite 400m runners in a 300m time trial. Though there was a significant peak in blood lactate concentration compared to placebo during recovery ($P<0.01$), no significant performance enhancement was reported between the CIT and placebo groups. The authors suggested that the higher blood lactate concentration, without the subsequent improvement in performance, may be due to an increased efflux via the pH contrast or due to a decreased uptake by working muscle. In the case of the latter, it was reasoned that H^+ may not be the main factor effecting rate limiting enzymes, but instead, “a change in the concentration of electrolytes which limit excitation contraction coupling would reduce the muscles’ ability to produce tension.” Therefore, alkalosis could lead to a decreased efflux of electrolytes from the cell, maintaining homeostasis and production of energy (Street et al. 2005). Furthermore, Zochowski et al. (2009) compared two unique administrations of Na-CIT (chronic and acute), each at the same dose of 0.3g/kg, to placebo in elite swimmers. Perhaps not surprisingly, considering the doses of Na-CIT administered, no significant treatment effects were found compared to placebo. It is important to note that this is the only study that has investigated the

potential effects of chronic ingestion of a lower level Na-CIT over a longer period of time to minimize the potential GI upset that follows in some cases of acute dosing.

There have been a few studies that analyzed the potential benefits of Na-CIT in long-term high intensity exercise with conflicting results (Kowalchuk et al. 1989, Oopik et al. 2003, Oopik et al. 2004, Potteiger et al. 1996, Schabort et al. 2000). Three of these studies (Oopik et al. 2003, Oopik et al. 2004, and Potteiger et al. 1996) found noteworthy effects. In a controlled environment, Oopik et al. (2003) found an improvement in 5km run performance ($P = 0.01$) on a treadmill at a dose of 0.5 g/kg taken 120min pre-performance. The same researchers, however, performed the same experiment as a field study and reported a lack of improvement between treatments, which they attributed to environmental factors, such as wind (Oopik et al 2004). Potteiger et al. (1996) recorded a 4% improvement in 30km cycling time ($P < 0.05$) and a significant increase in blood lactate concentration post-exercise ($P < 0.01$). There was no difference between treatments in power output over 30km; however, in the first 25min, the power output for the CIT trial was higher. Blood gas measurements were also recorded, but were not significantly different across treatments. According to the authors, these data are important for two reasons: “1) PO_2 similarity for the two trials indicates that oxygen delivery was consistent and the higher blood lactate concentration was not the result of a lack of oxygen, and 2) the lack of differences of PCO_2 indicates that any excess carbon dioxide formed as a result of buffering the excess H^+ , via carbonic anhydrase reaction, was ventilated” (Potteiger et al. 1996). In contrast, both

Schabert et al. (2000) and Kowalchuk et al. (1989), following different dosing protocols, reported a lack of improvement in both power output and time between treatments.

Research into the effect of alkalizing agents in females is limited and the results suggest that the effect is negligible. Two studies investigated the potential for an ergogenic effect with Na-CIT, and in both cases no significant differences in time were reported. The investigations chose well-trained runners, with Oopik et al. (2008) reporting 1500m run times and Tiriyaki and Atterbom (1995) assessing 600m run times on an outdoor track. Contrary to most other published research (Ibanez et al. 1995, Kowalchuk et al. 1989, Linossier et al. 1997, McNaughton 1990, Robergs et al. 2005, Oopik et al. 2003), blood lactate concentration was unchanged between trials. Although there are ethical considerations when administering a natural health product to women, more study should be done to elucidate the potential that alkalizing agents could have on women in sport.

Most of the studies mentioned above, however, have reported considerable limitations that may contribute to the ambiguous results found. First, most studies in well-trained athletes have a small sample size, resulting in a lack of power for adequate statistical analysis. Other confounding variables, such as wind speed, temperature, and altitude, may have also contributed to differences in results (Tiriyaki et al. 1995). Furthermore, in cases where improvement was minimal and non-significant, the argument can be made that any improvement in elite sport would be decisive in competition (Freiche et al. 2002). Second, CIT is an inhibitor of PFK, possibly inhibiting ATP production at higher doses (Kowalchuk et al. 1989, Oopik et al. 2008). In addition,

during longer-term high intensity exercise, factors such as hydration and electrolyte balance may also play significant roles in determining performance outcomes (Linossier et al. 1997). Third, administering Na-CIT with the correct pre-performance protocol is necessary in eliciting the potential ergogenic effects that the buffering agent may offer (McNaughton 1990, McNaughton & Cedaro 1992). Moreover, alkalizing agents may not be well tolerated, especially at higher doses, causing GI discomfort, which may be detrimental to performance (Schabert et al. 2000).

2.6.4 Intermittent Exercise

There are very few studies on repeated bouts of exercise with Na-CIT supplementation (Davidson et al. 2004, Cox & Jenkins 1994, Van Someren et al. 1998), and the results are not as convincing as those found when sodium bicarbonate is administered (McNaughton et al. 2008). A review done by McNaughton et al. (2008) summarized recent literature on the effect of sodium bicarbonate during multiple bouts of exercise and found 3 of 5 of the studies reported improvements in performance. In contrast, none of the three studies administering Na-CIT recorded any improvement (Table 1).

2.6.5 Effects on Cardio-Respiratory Performance

Cardio-respiratory performances tend to show a net benefit with Na-CIT supplementation (Frieche et al. 2002, Hauswirth et al 2005, Jain et al. 2003). Hauswirth

et al (2005) reported a significant improvement in muscle endurance during isometric knee extension in normoxia ($P < 0.05$), and a non-significant improvement in hypobaric hypoxic conditions (463mmHg, 61.7kPa). In a sport specific study, Fricke et al. (2002) reported a trend toward significance when cycling to exhaustion at 112% of $\text{VO}_{2\text{max}}$ in normoxia ($P = 0.09$). This represents an improvement of about 9s compared to placebo, which would be decisive in competition. In addition, significant improvements were reported in ventilation rate (VE) ($P < 0.018$), rate of elimination of CO_2 ($P < 0.0007$), and respiratory exchange ratio (RER) ($P < 0.01$) in both normoxia and hypoxia (2320m). These data suggest that the intake of an alkalizing agent attenuates ventilation rates and aids in elimination of CO_2 . It is possible that a central control prevails over peripheral regulatory mechanisms controlling VE, allowing a decreased VE during a state of increased pH due to alkalization by Na-CIT. Another possible explanation for the reported decrease in VE may be to a drop in sympathetic activity. However, research suggests that ingestion of an alkalizer does not alter sympathetic activity, during or post-exercise, compared to placebo (Bracken et al. 2005, Marx et al. 2002). Taken together, this is an important observation when considering the example of swimming where athletes must sustain a high rate of energy expenditure while suspending breathing for 20 - 30% of their race (Craig 1986).

2.6.6 Effects on Recovery

The recovery kinetics of acid-base balance is important when addressing training or sporting events requiring multiple sprint/recovery bouts. Robergs et al. (2005) observed that the recovery kinetics of acid-base balance have not addressed adequate durations of recovery (15min compared to 60min). This applies to sporting events, such as swimming or judo, where multiple performances are done during any given competition session, usually over a period of a couple hours. Therefore, the authors investigated blood pH, bicarbonate, and lactate recoveries comparing ammonium chloride, sodium bicarbonate, Na-CIT, and placebo. Consistent with the literature, peak blood pH, bicarbonate, and lactate concentrations, were observed in the alkalotic condition. Recovery was separated into two segments for pH: 1) 0 - 15min and 2) 15 - 60min. The first recovery segment revealed similar recovery slopes for all conditions, but the ammonium chloride and placebo trials had a significantly lower blood pH than the alkalotic trial ($P<0.05$). The second recovery segment followed a similar pattern with the alkalotic condition maintaining an overall higher pH. These findings were also reported by Jain et al. (2003). Furthermore, these authors investigated the recovery dynamics of oxygen deficit incurred at the onset maximal exercise. They reported that CIT reduced post-exercise oxygen debt incurred ($P<0.01$) following cycling at a set resistance to exhaustion.

2.7 Swimming

Competitive swimming provides an ideal model for the characterization of the anaerobic response and by extension alkalizing agents. Events range in duration from 22s (50m freestyle) to 15min (1500m freestyle) with the highest blood lactate concentrations found in the 200m (2min) event. Typical post-race blood lactate concentrations for these events are 6.4, 9.1, and 14.0mmol/L in the 1500m, 50m, and 200m events respectively (Vescovi et al. 2011). Furthermore, swimmers often race several events within 30 - 90min during any given session. Swimmers must also contend with restrictions placed on their breathing frequency during intense exercise, resulting in a unique interaction between muscle physiology, technique, and ventilation. Exercise hyperpnoea is limited during high intensity swimming because turning or lifting the head to breathe may jeopardize execution of proper stroke technique (Wells et al. 2005). During high intensity swimming, breath holding can result in significant decreases in the arterial partial pressure of oxygen (PaO_2), decreased blood pH, but an unchanged arterial partial pressure of carbon dioxide (PaCO_2) relative to non-frequency controlled breathing (PaO_2 unchanged, pH unchanged, PaCO_2 decreased) (Sharp et al. 1991). Indeed, swimming requires that the athlete sustain a high rate of energy expenditure and the suspension of breathing for approximately 20 - 30% of a race (Craig 1986). Given these limitations and the physiological consequences, it is likely that anaerobic metabolism is a significant contributor to metabolic power in competitive swimming, and may also be a primary determinant of fatigue and limitations in performance

(Noakes 2000). Therefore, administration of an alkalizing agent may potentially enhance performance by a greater magnitude than in cycling or running.

2.8 Side Effects of Sodium Citrate Ingestion

Both sodium bicarbonate and Na-CIT can induce to GI upset, cramps, or diarrhea and may not be tolerated well by all athletes. It has been suggested that approximately 10% do not tolerate these substances well (McNaughton et al. 2008). When Na-Cit is consumed, there is a possibility that these symptoms may be due to an increased osmolality of the GI tract and water may be shifted from the plasma to the intestine to counteract the hypertonicity (Requena et al. 2005). However, Na-CIT solutions are preferred to sodium bicarbonate. This is because, with a few exceptions (Cox and Jenkins 1994, Schobart et al. 2000), Na-CIT is associated with an improved GI acceptance profile. Nonetheless, there seems to be a direct relation between the appearance of these side symptoms and the dose administered, although high doses are needed to adequately modify the acid-base balance (McNaughton 1990). Attaining peak buffering potential, while minimizing the risk of GI distress before racing, is essential because performance may be negatively affected (Schabart et al. 2000). Pre-race experimentation is suggested as a prudent strategy as it could decrease the chance of the occurrence and severity of GI distress. In this respect, some researchers have experimented with allowing the intake of water *ad libitum* after supplying an alkalotic solution. While some studies have modified the administration of Na-CIT using capsules,

this presents the problem that a large number must be ingested.

2.9 Conclusion

In most cases, alkalizing agents seem to provide a benefit to sport performance, but the data on the effects of Na-CIT in high performance athletes is unclear. It was concluded by Carr et al. (2011), that the variability in response may be due to inconsistent methodology. Indeed, consistent adherence to a standard protocol has been an issue when investigating Na-CIT (Table 1). Nonetheless, the general consensus is that there is a performance enhancing effect with ingestion of Na-CIT. Therefore, more research with consistent methodology is recommended to elucidate the potential ergogenic effect of Na-CIT. In addition, there is a gap in the literature with respect to the study of young well-trained athletes who also participate in multi-event sports like swimming.

Table 1: A complete list of current literature pertinent to sodium citrate in sport and exercise as of February 2013

Author	N	Subjects	Gender	Exercise Mode	Duration	Dose (g/kg)	Load Time (min)	P-value (outcome variable)
Single Bout								
Ibanez et al. 1995	6	elite runners	male	outdoor track	300m	0.5	180	p>0.05 (35.43 ± 1.19, 35.55 ± 1.18s; CIT, PLC)
Kowalchuk et al. 1989	9	active students		cycle ergometer	20min 33%, 20min 66%, 95% VO2max to exhaustion	0.3	60	p>0.05 (310 ± 69, 313 ± 74s; CIT, PLC)
Linossier et al. 1997	5+3	moderately active students	male + female	cycle ergometer	120%VO2 peak to exhaustion	0.5	90	p<0.05 (297 ± 29, 258 ± 29s; CIT, PLC)
McNaughton & Cedaro 1992	10	active students	male	cycle ergometer	10, 30, 120, 240s	0.5	90	p<0.05 (Total Work and Peak Power: 120 and 240s; compared to Control and PLC)
McNaughton 1990	11	active students	male	cycle ergometer	60s	0.1, 0.2, 0.3, 0.4, 0.5	90	p<0.05, p<0.001, p<0.0001 (Total Work and Peak Power: 0.3, 0.4, 0.5; compared to Control and PLC)
Oopik et al. 2003	17	well-trained runners	male	treadmill	5km	0.5	120	p=0.01 (1153.2 ± 74.1, 1183.8 ± 91.4s; CIT, PLC)
Oopik et al. 2004	10	well-trained runners	male	outdoor field	5km	0.5	120	p=0.09 (1100.0 ± 79.1, 1082.7 ± 62.0s; CIT, PLC)

Author	N	Subjects	Gender	Exercise Mode	Duration	Dose (g/kg)	Load Time (min)	P-value (outcome variable)
Oopik et al. 2008	17	well-trained middle distance runners	female	outdoor track	1500m	0.4	90	p>0.05 (321.4 ± 26.4, 317.4 ± 22.5s; CIT, PLC)
Potteiger et al. 1996	8	well-trained cyclists	male	cycling ergometer	30km	0.5	90	p<0.05 (3459.6 ± 97.4, 3562.3 ± 108.5s; CIT, PLC)
Schabert et al. 2000	8	well-trained cyclists	male	Mounted personal bicycle	40km	0.2, 0.4, 0.6	60	p=0.886 (power output), p=0.754 (time)
Shave et al. 2001	7+2	elite athletes	male + female	outdoor track	3000m	0.5	60	p<0.05 (610.9 ± 36.6, 621.6 ± 31.4s; CIT, PLC)
Street et al. 2005	7	active	male	one leg knee extension	constant load (W) at 60RPM to exhaustion	0.3	150	p=0.1 (418 ± 57, 356 ± 23s; CIT, PLC)
Zochowski et al. 2009	7	elite swimmers		25m pool SC	200m	Chronic: 4 days x 0.3, Acute: 0.3	90	p>0.05 (125.8 ± 7.2, 125.4 ± 7.1, 125.7 ± 8.1; Chronic, Acute, PLC)

Author	N	Subjects	Gender	Exercise Mode	Duration	Dose (g/kg)	Load Time (min)	P-value (outcome variable)
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Multiple Bouts

Cox & Jenkins 1994	8	moderately active students	males	cycle ergometer	5 x (60s + 5min recovery)	0.5	90	(141.8 ± 6.9, 142.2 ± 6.2 KJ; CIT, PLC)
Davidson et al. 2004	9	recreational athletes		isokinetic knee extension/flexion 75°/sec	5x (10 reps + 1min recovery)	0.3	75	Total work set one/five: 312/221, 287/200 KJ; CIT, PLC
Van Someren et al. 1998	9 + 3	active non-athletes	male + female	cycle ergometer	5 x (45s + 1 min recovery)	0.3	90	p>0.05 (Total Work and Peak Power)

Oxygen Debt

Jain et al. 2003	10	untrained	male	cycle ergometer	2kg friction fixed pedal rate to exhaustion	0.5	120	p<0.01 (O2 debt: 4.56 ± 0.66, 7.77 ± 0.96L; CIT, Control)
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Author	N	Subjects	Gender	Exercise Mode	Duration	Dose (g/kg)	Load Time (min)	P-value (outcome variable)
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Hypoxia

Freiche et al. 2002	17	students	male	cycle ergometer	112% VO2max at No or H (2320m)	0.4	120	p<0.018 (VE lower), p<0.0007 (VCO2 higher), p<0.0119 (RER higher), with CIT in both No and H.
Hauswirth et al. 1995	8	healthy	male	isometric knee-extension	35% MVC at No or HH (463mmHg, 61.7kPa)	0.4	120	p<0.05 (muscle endurance: 192 ± 17, 156 ± 17; No + CIT, No + PLC) Non-significant increase in endurance for HH (168 ± 15, 145 ± 10; HH + CIT, HH + PLC)

Catecholamine Response

Bracken et al. 2005	8	healthy	male	cycle ergometer	2min 110% VO2max	0.5, 0.3	60	Plasma [DA], [NE], and [EPI] were unaltered with CIT
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Author	N	Subjects	Gender	Exercise Mode	Duration	Dose (g/kg)	Load Time (min)	P-value (outcome variable)
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Diet Manipulation

Ball & Maughan 1997	6	healthy	male	cycle ergometer	100% VO ₂ max to exhaustion	Normal or h-CHO diet: 3days + 0.3 CIT	180	No difference: 202 ± 23, 201 ± 16, 205 ± 24, 204 ± 12s; N + CIT, h-CHO + CIT, Normal + PLC, h-CHO + PLC
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Fluid and Electrolyte Regulatory Hormones

Oopik et al. 2010	13	well-trained runners	male	treadmill	running test to exhaustion	0.5	120	p=0.003 (ALD lower in CIT post ingestion)
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Recovery

Robergs et al. 2005	12	well-trained cyclists	male	cycle ergometer	110% VO ₂ max to exhaustion	0.3 NH ₄ Cl vs. 0.2 + 0.2 NaHCO ₃ + CIT	60	p<0.05 (improved pH recovery), p<0.05 (post exercise acidosis: pH 7.15 ± 0.06, 7.21 ± 0.007, 7.16 ± 0.06; NH ₄ Cl, NaHCO ₃ +CIT, PLC)
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Author	N	Subjects	Gender	Exercise Mode	Duration	Dose (g/kg)	Load Time (min)	P-value (outcome variable)
Comparison								
Parry-Billings & MacLaren 1986	6	active students	male	cycle ergometer	3 x (30s Wingate Tests + 6min recovery)	0.3 NaHCO ₃ , NaHCO ₃ + CIT, CIT, NaCl	150	Total work over 3 tests 103%, 102%, 101%; CIT, NaHCO ₃ + CIT, NaHCO ₃ over PLC
Potteiger et al. 1996	7	well-trained runners	male	treadmill	30min at lactate threshold → 110% to exhaustion	0.3 NaHCO ₃ vs. 0.5 CIT	120	287 (SEM 47.4), 172.8 (SEM 29.7), 222.3 (SEM 39.7)s; NaHCO ₃ , CIT, PLC
Tiryaki & Attebom 1995	11+4	well-trained track athletes and trained non-athletes	female	outdoor track	600m	0.3 NaHCO ₃ , 0.3 CIT	150	(121.5, 119.9, 120.4s; NaHCO ₃ , CIT, PLC)
Van Montfoort et al. 2005	15	well-trained endurance runners	male	treadmill	exhaustion ~80s	0.3 NaHCO ₃ , 0.525 CIT, 0.4 NaLac, 0.209 NaCl	90	NaHCO ₃ :NaCl 2.7% improvement; CIT:NaCl 0.5% improvement. (82.3, 78.2, 80.2, 120.4s; NaHCO ₃ , CIT, Lactate, PLC)

ALD = Aldosterone
CIT = Sodium citrate
DA = Dopamine
EPI = Epinephrine
H = Hypoxia
HH = Hypobaric hypoxia
h-CHO = High carbohydrate
NaLac = Sodium lactate
Lac = Lactate
MVC = Maximal voluntary contraction
NaCl = Sodium chloride
NaHCO₃ = Sodium bicarbonate
NH₄Cl = Ammonium chloride
NE = Norepinephrine
No = Normoxia
PLC = Placebo
RER = Respiratory exchange ratio
VE = Ventilation

Chapter 3: Methods

3.1 Participants

Ten, well-trained, male, adolescent swimmers (mean \pm SEM, age 14.9 ± 0.4 years; body mass 63.5 ± 4 kg) participated in the current study. Participants were recruited through team announcements from competitive swimming clubs in the area. A letter of invitation was handed out to participants that fit the study population inclusion criteria (male swimmers who have achieved at least regional standards). A study investigator was there to answer questions. Participants (and their parent or guardian) provided written informed assent and consent. Participants were asked to refrain from abnormal, energy consuming lifestyle choices between the four swimming trials. Participants refrained from using caffeine and alcohol during the day of each trial. All procedures were approved by Health Canada Natural Health Products Directorate and Brock University Research Ethics Board (Protocol: 12-009).

3.2 Experimental Design

The current study was performed using a randomized, double-blinded, placebo controlled, cross-over design. All participants performed four swimming trials under four treatment conditions determined by the amount of, and time over which, sodium citrate dihydrate was ingested. Specifically, all participants randomly performed 2

experimental trials: 1) acute (ACU), 2) chronic (CHR), and 2 placebo trials: 3) acute placebo (PLC-A), and 4) chronic placebo (PLC-C). The participants were randomly assigned by computerized random number generator. The study was conducted during the mid-season period of training in a 4 week period without a competition to minimize training and tapering effects. Each Na-CIT trial was separated by at least a 6 day washout period.

3.3 Sodium Citrate Dihydrate and Placebo Loading Protocol

The placebo and Na-Cit were delivered in a solution of 500mL of Crystal Light Pink Lemonade flavoured water. Ten adult volunteers tested multiple flavours to find an optimal masking flavour of Na-CIT in an effort to ensure blinded supplementation. The ACU involved taking 0.5g/kg of body mass of Na-CIT in solution with 500mL of flavoured water 120min pre-performance. The CHR involved taking 0.1g/kg of body mass of Na-CIT three times per day for 3 days and on the 4th day a dose of 0.3g/kg of body mass, 120min pre-performance. The PLC-A and PLC-C had 500mL of flavoured water in equal amounts and frequency as their corresponding experimental trial. The placebo and Na-Cit bottles were coded by an independent researcher, and the key was used only at the time of data analysis at the end of the study by the primary researcher. During every trial, swimmers were asked anecdotally if they knew which solution they were ingesting and if they were experiencing any GI discomfort.

3.4 200m Swim Trials

The 200m swim trials were conducted at Brock University in a short-course (25m) pool. Each participant swam a 200 metre long event in a stroke of their choosing at maximal effort each trial day. The choice of stroke was given to increase participant motivation and create a near real life setting. For each swimmer, the same stroke was used for all four trials (backstroke n = 1, breaststroke n = 2, freestyle n = 7, individual medley n = 1). All swimmers wore their regulation competition apparel for each trial. Warm-up and warm-down procedures were based solely on each swimmer's typical regimen for competition. Every trial was done during the same time of the day (5:00 - 6:00pm) in order to minimize diurnal and daily variations. The 200m swim began with a dive from the blocks initiated by the same starter's "take your marks, and go." Swimmers' times and rates of perceived exertion (RPE) were recorded at the end of each trial. All performance times were recorded with a manual stopwatch by the same investigator.

3.5 Blood Sampling and Analysis

Blood was collected pre-ingestion, 100min post-ingestion (20min pre-trial), and 3min post-trial. A venous blood sample was collected by finger prick and analyzed immediately by an automated lactate analyzer (Arkay Lactate Pro LT-1710) to determine whole blood lactate. Blood lactate has been measured following post-exercise at 0min, 2min, 3min, 5min, and 10min time points with the highest

concentrations recorded between 3 - 5min post-exercise (Vescovi et al. 2011, Frieche et al. 2002, Bangsbo et al. 1994, Rimaud et al. 2010). With the same finger prick, ~95µL blood was collected into a plain capillary tube and immediately analyzed for base excess (BE), pH, bicarbonate, and PCO₂ by i-STAT 1 Analyzer (Abbott Point of Care). Blood collection was done by the same two researchers for each trial.

3.6 Statistical Analysis

The approach to the calculation of sample size depends on the complexity of the study design. Using traditional statistics, sample size is calculated from known measures of central tendency and variability related to an expected difference that has biological significance. The only factor in these cross-sectional study designs that can be manipulated to increase power is the sample size. However, with repeated measurements, such as in the present study, it is possible to take into account the repeated observations within an individual in the sample size estimate. Increasing the number of repeated observations will reduce the required sample size because the within individual variance is reduced. In practice, when looking for a given absolute difference, smaller sample sizes are therefore needed for repeated measures studies compared with cross-sectional studies. With this in mind, we used Cohen's *d* (Cohen 1992) of moderate to large effect sizes ($d = 0.65-1.02$), along with the pre- and post-trial blood lactate measurements from a published 5 km run study in adults (Oopik et al. 2003) to detect between trial differences with a power of 0.80 and alpha at 0.05. This

resulted in a sample size of 8. It would, therefore, seem reasonable to recruit 10 subjects to allow for a 10% drop out rate, yet maintaining adequate power.

The Statistical Package for Social Sciences (SPSS Inc., Version 19.0) was used for data analyses and statistical significance was accepted at $p < 0.05$. Time and lactate concentrations were analyzed with paired t-tests for direct desired comparisons. RPE was analyzed with a repeated measures ANOVA to establish equal effort between all trials. Due to data collection issues, leading to a few missing data points, BE, bicarbonate, pH, and PCO_2 were analyzed as a between groups ANOVA and the assumption of equal sample sizes was not satisfied. This was accounted for in simple comparisons by using a Gabriel's *post-hoc* when the ANOVA yielded a significant effect.

Data were analyzed for the potential of finding "responders" and "non-responders." If participants had a greater than 0.4% improvement, which corresponds to a significant competitive improvement, in ACU versus PLC-A, then they were considered responders. The 0.4% improvement in swimming performance was derived by analyzing the magnitude of improvement needed to medal at the Olympics when a swimmer is ranked in the Top 10 in the World (Pyne et al. 2004, Trewin et al. 2004). Estimating the practical significance of observed effects overcomes a potential limitation of statistical significance where an outcome is assessed as non-significant ($P > 0.05$) but is of sufficient magnitude to be practically or clinically important.

Of the ten swimmers, five were identified as responders. Anthropometric data were compared between responders and non-responders for differences in age and body mass using an independent sample T-test. Due to small sample size, responders

did not satisfy the assumptions of normality for time and lactate concentrations and were analyzed with a non-parametric Wilcoxon Signed Ranks test. Lactate concentrations of responders and non-responders were compared using a Mann-Whitney U test.

Chapter 4: Results

4.1 200m Swim Trials

Anthropometric data are presented in Table 2. The PLC-A and PLC-C trials had the same mean performance times (143.5 ± 4.7 , and 143.5 ± 5.4 s for PLC-A and PLC-C, respectively) proving that the young swimmers were able to accurately reproduce their performance. Mean performance time was also not different across the four trials (143.5 ± 4.7 , 143.9 ± 5.4 , 143.7 ± 5.4 , 143.5 ± 5.4 s for PLC-A, ACU, CHR, PLC-C, respectively). When comparing PLC-A versus ACU, PLC-C versus CHR, and ACU versus CHR no significant differences were found ($P = 0.62$, 0.89 , and 0.75 , respectively) (Fig. 1). Furthermore, RPE was not statistically different across all trials ($F = 0.75$, $P = 0.53$, $\eta^2 = 0.77$) (Fig. 2).

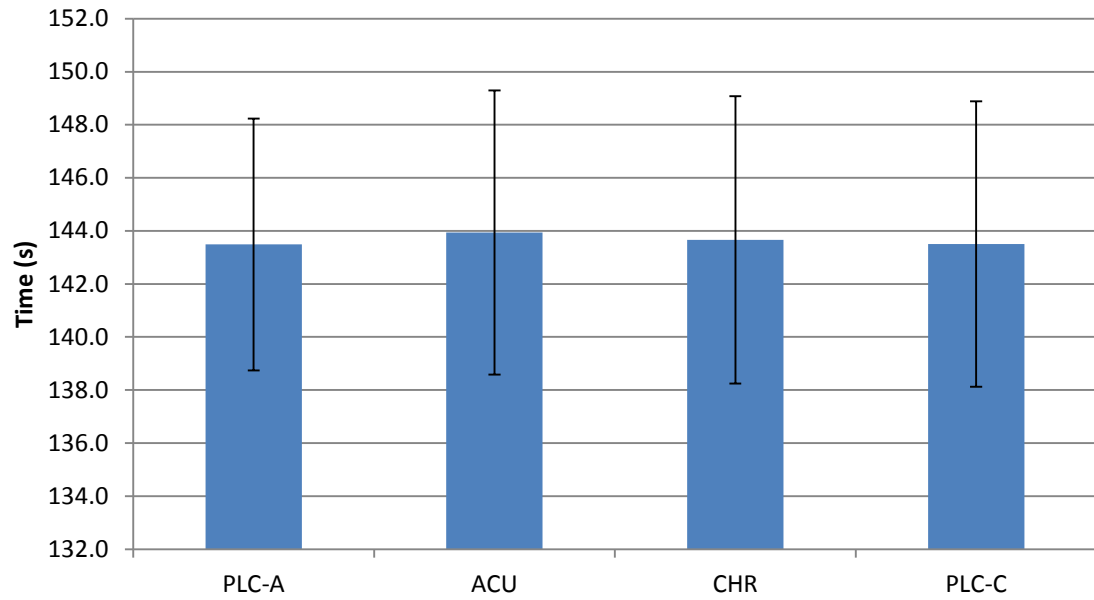


Figure 1: Mean \pm SEM 200m swimming time of the 10 participants for each trial.

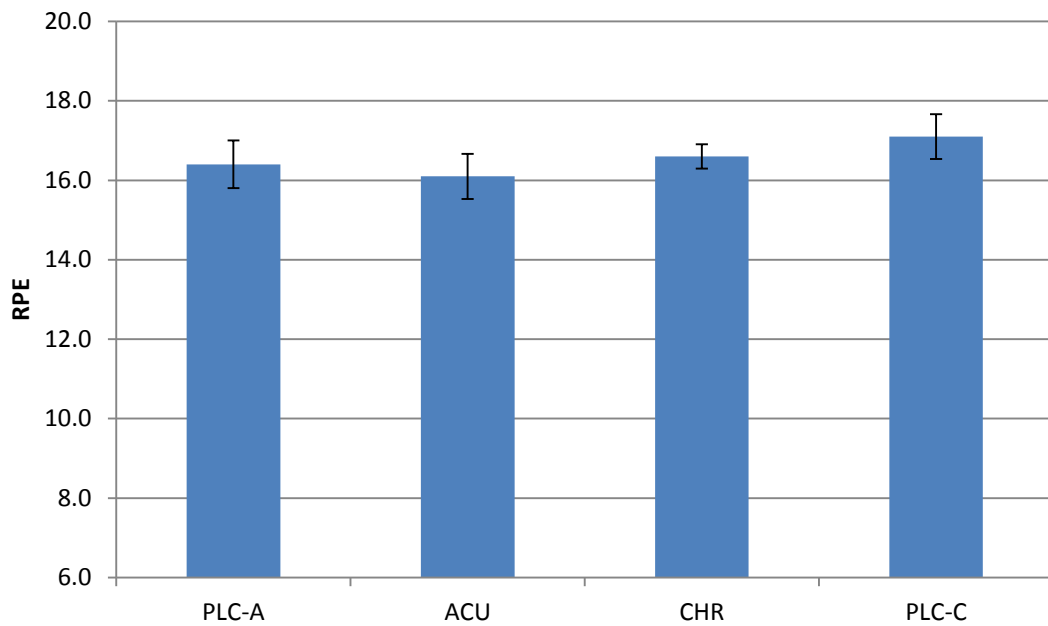


Figure 2: Mean \pm SEM RPE of the 10 participants for each trial.

Data were analyzed for the potential of finding “responders” and “non-responders.” Of the ten swimmers, five were identified as responders (136.4 ± 7.4 ,

135.0 \pm 7.6, 135.2 \pm 7.2, 136.2 \pm 8.7 for PLC-A, ACU, CHR, PLC-C, respectively) with a significant improvement of 1.03% in the ACU compared to the PLC-A trial ($P = 0.048$) (Fig. 3). However, there were no significant differences when comparing CHR versus PLC-C ($P = 0.89$) and ACU versus CHR ($P = 0.2$). There was no significant ergogenic or ergolytic effect found in the non-responders. Responders were characterized with a non-significant higher mean age (15.4 vs. 14.4yrs) ($P = 0.27$) and body mass (67.4 vs. 59.3kg) ($P = 0.33$) compared to the non-responders (Table 2).

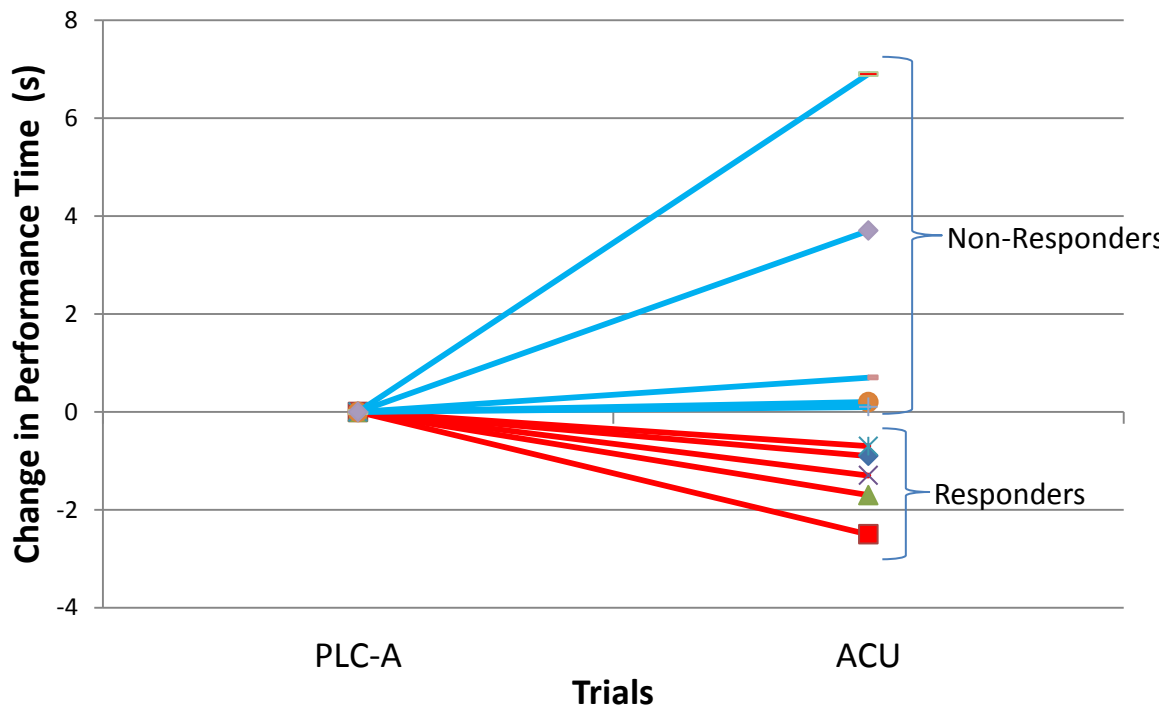


Figure 3: Absolute change in performance time for the responders ($n = 5$) and non-responders ($n = 5$) comparing acute (ACU) versus acute placebo (PLC-A) supplementation trials. Performance was significantly different in the ACU versus PLC-A ($P = 0.043$). Each *line* represents a different swimmer.

Table 2: Physical characteristics (mean \pm SEM) of all 10 participants, 5 responders and 5 non-responders.

	Age (yrs)	Body Mass (kg)	BMI (kg/m ²)
All	14.9 \pm 0.4	63.5 \pm 4.0	21.0 \pm 0.6
Responders	15.4 \pm 0.5	67.4 \pm 4.1	22.1 \pm 1.1
Non-Responders	14.4 \pm 0.4	59.3 \pm 3.8	19.8 \pm 0.6

4.2 Lactate Concentrations

As expected, lactate concentration was significantly different from basal or post-ingestion to post-trial ($P = 0.0001$) across all trials. There were no significant differences post-race values between trials ($P = 0.1, 0.26, 0.31$: PLC-A versus ACU, CHR versus PLC-C, ACU versus CHR) (Fig. 4). However, the responders had significantly increased lactate concentrations in the ACU versus PLC-A trial, but this was not the case when comparing the CHR versus PLC-C and ACU versus CHR trials ($P = 0.048, 0.345, 0.325$, respectively). Furthermore, responders had significantly higher lactate concentrations than non-responders in the ACU and the CHR trials ($P = 0.016, P = 0.047$, respectively) (Fig. 5).

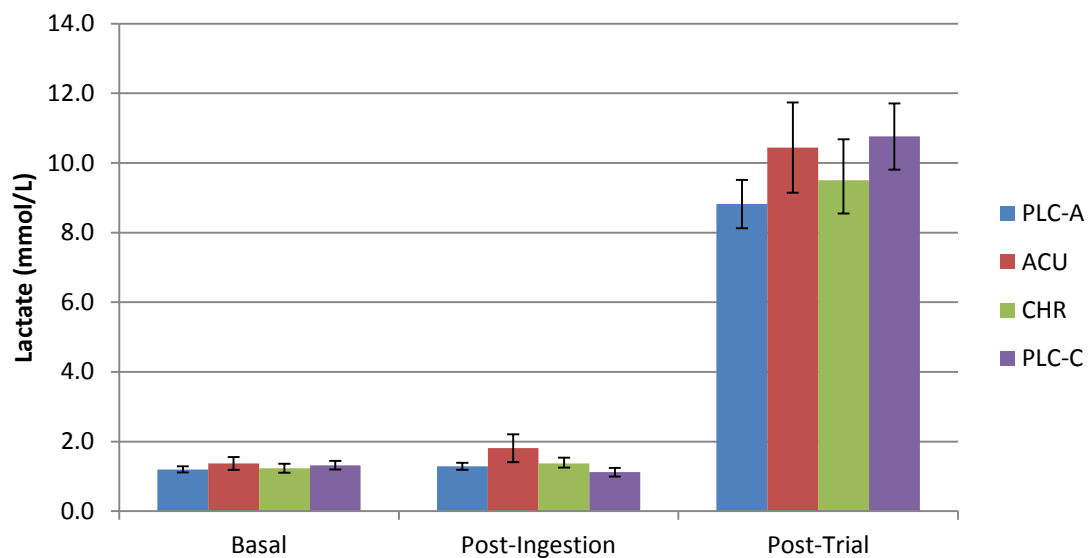


Figure 4: Mean \pm SEM lactate concentration (mmol/L) of the 10 participants following basal, post-ingestion, and post-trial time points of each trial.

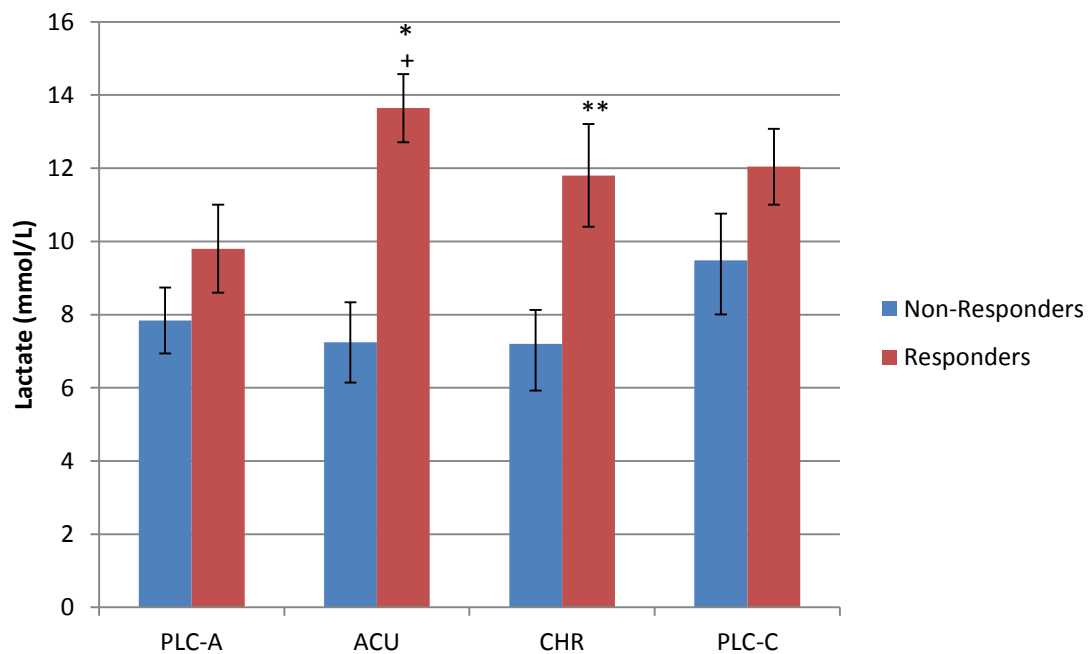


Figure 5: Mean \pm SEM post-trial lactate concentration (mmol/L) of responders and non-responders. *Significantly different ($P = 0.043$) from acute placebo trial (PLC-A). +Significantly different ($P = 0.016$) from non-responders in the ACU trial. **Significantly different ($P = 0.047$) from non-responders in the CHR trial.

4.3 Physiological Response to Na-CIT

BE, bicarbonate, and PCO_2 were analyzed for differences between basal, post-ingestion, and post-exercise. Simple effects for BE indicated significant differences between conditions for basal and post-ingestion values, but not for post-exercise values ($F_{(3,21)} = 3.3$ $P = 0.04$; $F_{(3,18)} = 21.6$, $P = 0.0001$; $F_{(3,14)} = 1.14$ $P = 0.37$: respectively). *Post-hoc* analysis revealed that BE in the CHR trial was significantly higher than in the ACU trial ($P = 0.05$). As was expected, BE was significantly higher in the ACU versus PLC-A trial and in the CHR versus PLC-C trial ($P = 0.0001$, $P = 0.001$, respectively). This did not lead to an attenuation in the BE decrease post-exercise compared to placebo (Fig. 6).

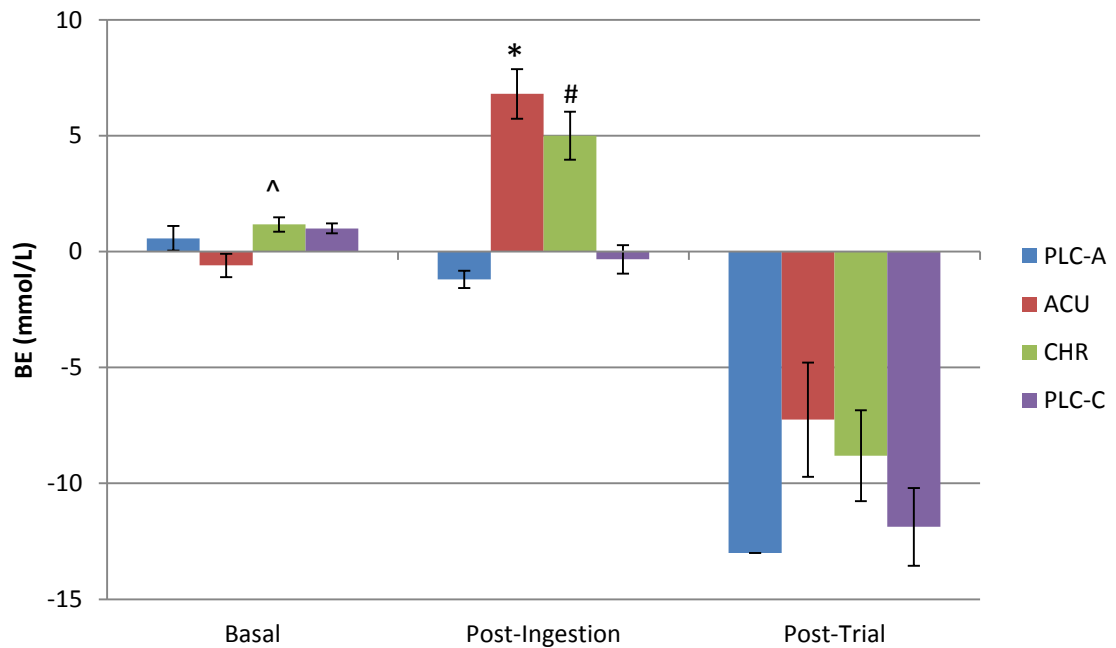


Figure 6: Mean \pm SEM base excess (BE) (mmol/L) following basal, post-ingestion, and post-trial time points of each trial. [^]Significantly different ($P = 0.05$) from the acute trial (ACU). ^{*}Significantly different ($P = 0.0001$) from the acute placebo trial (PLC-A). [#]Significantly different ($P = 0.001$) from the chronic placebo trial (PLC-C).

As was expected, bicarbonate concentration was found to be significantly different post-Na-CIT ingestion ($F_{(3,21)} = 2.13, P = 0.13$; $F_{(3,18)} = 20, P = 0.0001$; $F_{(3,14)} = 1, P = 0.43$: basal, post-ingestion, post-exercise). Na-CIT ingestion significantly increased bicarbonate levels in both the ACU and CHR treatment conditions compared to their corresponding placebo ($P = 0.0001, P = 0.002$, respectively). Although bicarbonate levels significantly increased post-exercise within each treatment condition compared to placebo, there was no significant difference between conditions (Fig. 7). Simple effects for pH indicated a significant increase for post-ingestion values ($F_{(3,19)} = 1.12, P = 0.367$; $F_{(3,15)} = 5.14, P = 0.012$; $F_{(3,14)} = 1.64, P = 0.224$: basal, post-ingestion, post-exercise); however, *post-hoc* analysis results indicated a trend to significance when comparing the ACU trial to the PLC-A trial ($P = 0.06$) (Fig 8). Furthermore, PCO_2 values were not significantly changed between conditions ($F_{(3,21)} = 1.6, P = 0.22$; $F_{(3,18)} = 1.9, P = 0.16$; $F_{(3,14)} = 0.19, P = 0.9$: basal, post-ingestion, post-exercise) (Fig. 9).

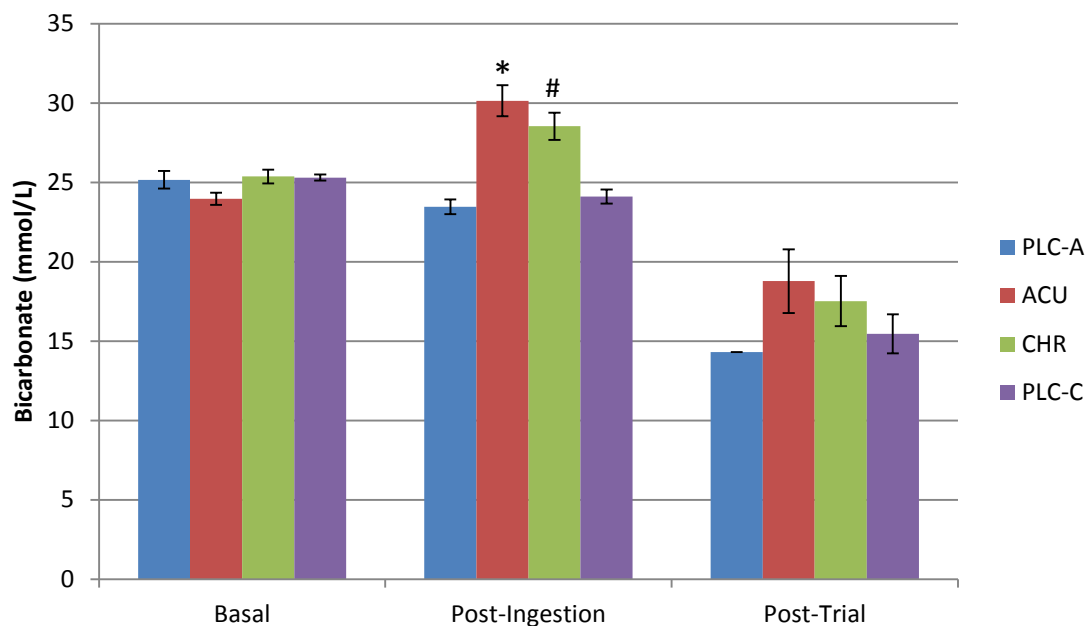


Figure 7: Mean \pm SEM bicarbonate concentration (mmol/L) following basal, post-ingestion, and post-trial time points for each trial. *Significantly different ($P = 0.0001$) from the acute placebo trial (PLC-A). #Significantly different ($P = 0.002$) from the chronic placebo trial (PLC-C).

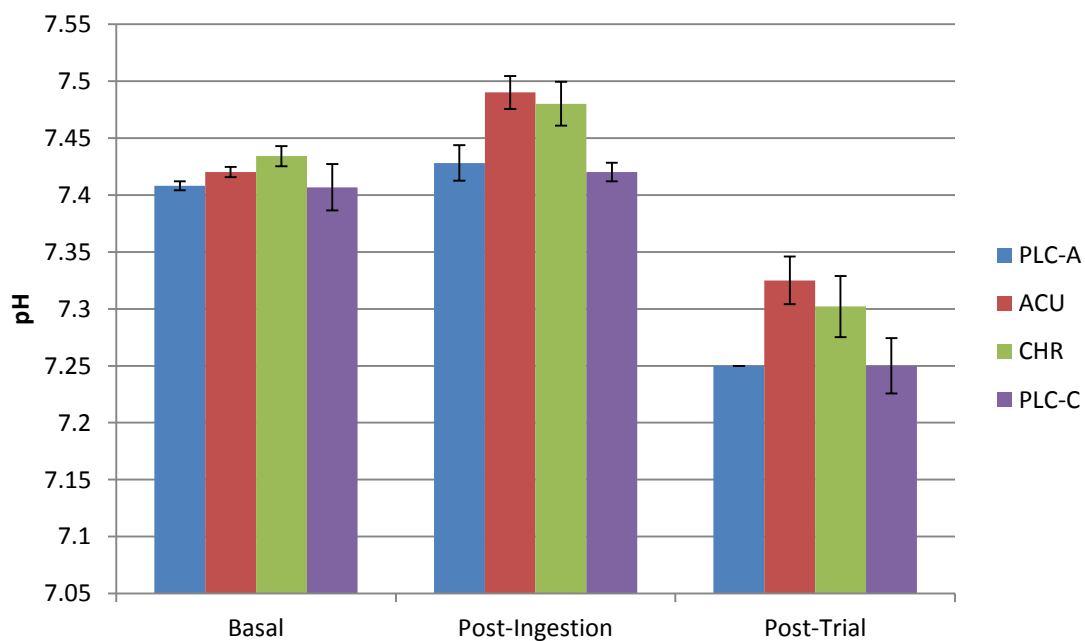


Figure 8: Mean \pm SEM pH following basal, post-ingestion, and post-trial time points for each trial.

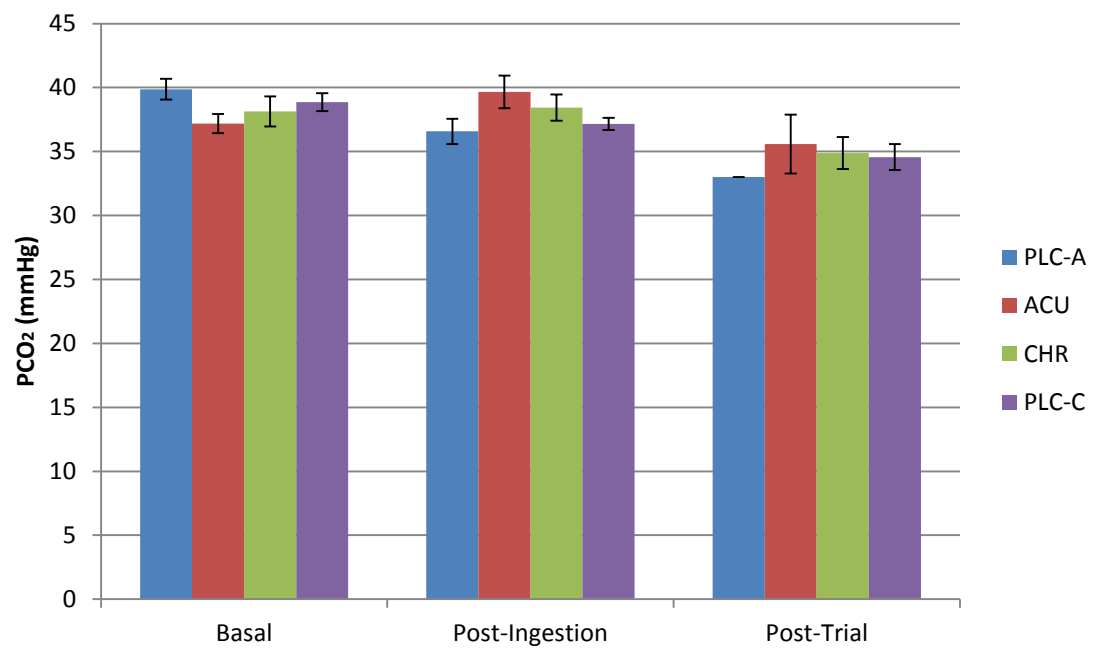


Figure 9: Mean \pm SEM PCO_2 (mmHg) following basal, post-ingestion, and post-trial time points for each trial.

Chapter 5: Discussion and Conclusions

5.1 Discussion

In this study, well-trained swimmers performed four 200m time trials at maximal effort, under different treatment conditions. Na-CIT was given for two dosing protocols (ACU and CHR), each with a corresponding placebo (PLC-A and PLC-C). When swimmers were asked if they knew which solution they were ingesting the response was often that they did not know (data not shown). This is the first study that we know of to apply a chronic dosing regimen to Na-CIT in an effort to improve performance and minimize GI discomfort. Each swimmer was asked if any GI discomfort occurred throughout the study and none was reported (data not shown). Chronic dosing of alkalizing agents was first employed by (Joyce et al. 2012) with sodium bicarbonate in an effort to elicit an ergogenic effect while minimizing GI upset, which often occurs with acute dosing protocols. Furthermore, this is also the first study that we know of to investigate the potential ergogenic effects of Na-CIT in adolescent athletes. Swimmers are often young when they reach elite level competition. The 2012 Olympics reported that among the swimming medalists, twenty-five were under 21yrs. and eight were under 18yrs. Therefore, investigating ergogenic aids in young competitive swimmers is relevant, and until now, lacking in the literature.

The 200m performance times were not improved with Na-CIT ingestion in either ACU versus PLC-A or CHR versus PLC-C. Na-CIT is postulated to work predominantly as

an alkalizing agent; however, more study is needed on its intracellular effects. Lactate facilitation out of working muscle is increased under alkalotic conditions during and post-exercise compared to placebo (Robergs et al. 2005, Sostaric et al. 2006). However, post-trial lactate concentrations were also not statistically different between trials. The literature is predominantly in agreement: lactate concentrations are significantly higher post-exercise with Na-CIT ingestion compared with control or placebo (Linossier et al. 1997, McNaughton 1990, McNaughton & Cedaro 1992, Oopik et al. 2003, Shave et al. 2001), even when performance outcomes are not improved with supplementation (Ibanez et al. 1995, Kowalchuk et al. 1989, Oopik et al. 2003). Therefore, a higher lactate concentration post-trial, with Na-CIT ingestion, was expected.

Interestingly, when factoring the age of the sample population of this study, a possible explanation emerges. Children and adolescents have been found to have age-related divergences in muscle metabolism compared to adults (Beneke et al. 2005). It is well established that energy provision from anaerobic glycolysis is lower in children than adults during high-intensity exercise (Boisseau & Delamarche 2000, Ratel et al. 2002). The difference may be explained by several factors: i) reduced activity of PFK (Boisseau & Delamarche 2000, Eriksson et al. 1973, Falk & Dotan 2006, Fournier et al. 1982), ii) lower activity of lactate dehydrogenase (Boisseau & Delamarche 2000, Eriksson et al. 1973, Falk & Dotan 2006, Fournier et al. 1982), iii) limited ability to recruit and use higher-hierarchy motor units possibly due to neuromuscular immaturity (Dotan et al. 2012, Falk & Dotan 2006), and iv) a greater reliance on aerobic oxidative enzymes for energy provision (Beneke et al. 2005, Boisseau & Delamarche 2000, Falk & Dotan 2006).

Furthermore, this difference may be the reason for the smaller intramuscular pH change and lower lactate concentration found in children and adolescents after maximal exercise compared to adults (Beneke et al. 2005, Dotan et al. 2003, Falk & Dotan 2006, Eriksson et al. 1973, Ratel et al. 2002). While training can affect these mechanisms and shift the response to that of an average adult (Fournier et al. 1982) the population of this study were regional and national level qualifiers and therefore do not train at an elite capacity. Taken together, these findings provide a possible explanation for the lack of a significant improvement in swim time and change in lactate concentration post-trial.

The magnitude of the response to Na-CIT was not consistent across all participants. Similar to the potential difference in physical maturity level found in adolescents 13 – 17 years of age, a difference in performance was found in response to plasma alkalization. Given the age-related divergences in muscle metabolism, we investigated the potential to find participants that respond to Na-CIT at a greater magnitude than others. Therefore, the data were analyzed for responders and non-responders. Responders were chosen if they had greater than 0.4% improvement, which corresponds to a significant competitive improvement (Pyne et al. 2004, Trewin et al. 2004), in ACU versus PLC-A. From the sample population, there were five responders and five non-responders. Interestingly, responders were characterized with a higher mean age, body mass and BMI compared to non-responders (Table 2); however, this did not reach statistical significance.

The responders had a significant 1.03% mean performance improvement in the ACU trial, but not in the CHR trial. Similarly, post-trial lactate concentrations were higher in the ACU trial, but not in the CHR trial. When compared to non-responders, responders had higher post-trial lactate concentrations in both the ACU and CHR trials. In fact, Na-CIT did not induce any ergogenic or ergolytic effect in non-responders and they did not attain typical blood lactate concentrations after the 200m time trials, as was observed for the responders. Therefore, those who developed higher post-trial lactate levels benefited from the acute supplementation. These findings are supported by the established age-related divergences in muscle metabolism and provide further evidence on the effectiveness of Na-CIT supplementation in the older adolescent swimmers who make up the group of responders.

Paramount for coaches and athletes is the magnitude of worthwhile performance enhancement that can be gained from an ergogenic aid. A key limitation to sport specific studies, especially when studying elite athletes, is finding a worthwhile improvement in performance while coinciding with a statistically significant improvement. Statistical significance at $p < 0.05$ can sometimes be too stringent with respect to finding a meaningful improvement in performance. The argument can be made that any improvement in elite sport would be welcomed by coaches and athletes. Indeed, the smallest worthwhile change for competition performance is 0.4% in Olympic level swimmers when comparing finalists to medalists (Pyne et al. 2004, Trewin et al. 2004). This corresponds to ~ 0.25 s in 100m events and ~ 0.5 s in 200m events (Anderson et al. 2008). Although, the performance improvement in the ACU trial was statistically

significant; an agreement should be made that when testing competitive athletes, minimum competitive significance should be established, independent of statistical significance.

The effect on both swimming performance and plasma alkalization was dependent on the supplementation protocol. The ACU protocol benefited the performance of the responders; however, CHR protocol did not yield any significant improvement or increase lactate concentration. The CHR protocol was enacted to incrementally increase plasma BE over a longer time period to allow similar blood alkalization with a smaller dose 120min pre-trial. The rationale behind the novel dosing method was to minimize potential for performance inhibiting GI upset. Perhaps the CHR dose pre-trial was insufficient to elicit performance enhancement, even with the chronic dosing protocol over the previous three days. Another factor may be the period of time between the last chronic dose and the pre-trial dose of Na-CIT. Optimally, the pre-trial dose would have been the morning after the last chronic dose; however, the swims were performed in the late afternoon. Further experimentation with the timing of the last chronic dose and the pre-trial dose may be necessary to find an optimal protocol, should one exist. On the other hand, BE was significantly increased at the basal time point comparing CHR versus ACU. Whether this is due to physiological variations or an actual effect of the dosing protocol cannot be proven as no measurements were made to track the kinetics of Na-CIT during the CHR dosing period. The increase in BE at the basal time point did not coincide with an increase in bicarbonate at the same time point. PCO_2 was unchanged between and within conditions. Therefore, we can conclude that

the change in BE was due to the change in bicarbonate and not due to respiratory changes. BE reflects only the non-respiratory component of pH disturbances in extracellular fluid; therefore, the lack of a significant increase in bicarbonate is unknown. Plasma BE and bicarbonate concentration were in line with the values reported in the literature post-ingestion (Kowalchuk et al. 1989, Linossier et al. 1997, Street et al. 2005). Both the ACU and CHR trials resulted in significant increases in BE and bicarbonate; however, this did not translate to a comparable difference post-trial.

5.2 Conclusion

In this double-blind, placebo controlled, cross-over trial Na-CIT supplementation showed a significant ergogenic in the adolescent swimmers. More, specifically, acute supplementation of Na-CIT provided sufficient alkalization for performance enhancement in 200m time trials in responders who were older and in the later stages of physiological maturity. Post-trial lactate concentrations were also higher for this group. However, the novel chronic supplementation protocol was not significantly advantageous for responders and non-responders. Although it did provide a significantly increased BE and bicarbonate concentration post-ingestion, this increase was not as significant as what was measured in the ACU supplementation. Adequate timing of the CHR dosing before the trial day may have been a factor in the lead up to the basal time measurement. Therefore, the null hypotheses of no differences in 200m performance time between the ACU and PLC-A, the CHR and PLC-C, and the ACU and CHR trials are

accepted. However, when considering possible age related divergences in muscle metabolism, amongst responders the null hypothesis of no differences in 200m performance time between the ACU and PLC-A trials is rejected.

5.3 Study Limitations and Recommendations

The small sample size was the main limiting factor of this study, which may have resulted in the lack of significance. In addition, due to laboratory equipment malfunction, not all samples of BE, bicarbonate, and PCO_2 were completed. Therefore, there was not an equal sample size between groups when the variables were analyzed. The literature reports that bicarbonate concentration post-exercise is still significantly higher with an equal drop compared to placebo (Robergs et al. 2005). This higher bicarbonate concentration post-exercise allows for an improved rate of recovery. Perhaps the slight increase in bicarbonate post-exercise balances the recovery kinetics in favour of the alkalotic condition. Acidosis affects pH sensitive transport proteins and glycolytic mechanisms, which in turn affects cell homeostasis. While these may be factors in the improved recovery kinetics, this study was not able to definitively prove this hypothesis.

The within-athlete design used in the present study poses some unique challenges to researchers. In sport, it is often difficult to include a control group in the experimental design. Often sample size is not large enough for adequate statistical power to test all desired variables (Table 1), as was the case in this study. Confounding

variables are numerous and pose a risk to researchers attempting multi-day trials over a long period of time. Factors such as the training cycle of the participants may change, or training effects on performance can cause skewed results. Furthermore, psychological performance factors or physical health can detrimentally affect performance outcomes.

The analysis comparing responders to non-responders was an unpredicted observation during the inception of the study. As a result, characterization of each group of participants can only be made through age and body mass. Responders were characterized as being older and having a higher body mass than non-responders. This suggests that they were potentially in the later stages of physical maturity; however, sexual maturation was not measured. Furthermore, lean body mass was not measured, which may have been a more useful indicator comparing responders to non-responders than body mass alone.

5.4 Future Research

In future studies, where Na-CIT supplementation is used among participants with wide ranges of body mass, a new dosing method may be employed to minimize potential for fluid retention. This has been considered a potential hindrance to performance when ingesting an alkalizer in a dissolved solution (Oopik et al. 2004). Future protocols should adjust the volume of fluid, at a standard dose, to drink per body mass instead of a standard fluid volume of 500mL for an adjusting dose of Na-CIT per body mass. This way each participant would consume a volume of fluid according to their body mass in order

to control for the potential of changes in body mass and fluid retention of participants with different body masses.

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Appendices

Appendix 1

Ratings of Perceived Exertion

6	
7	VERY, VERY LIGHT
8	
9	VERY LIGHT
10	
11	FAIRLY LIGHT
12	
13	SOMEWHAT HARD
14	
15	HARD
16	
17	VERY HARD
18	
19	VERY, VERY HARD
20	

Appendix 2

Brock Research Ethics Board Letter of Approval



Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: 2/6/2013
PRINCIPAL INVESTIGATOR: KLENTROU, Nota - Kinesiology
FILE: 12-009 - KLENTROU
TYPE: Masters Thesis/Project STUDENT: Colin Russell
 SUPERVISOR: Nota Klentrou
TITLE: Effects of acute versus chronic closing of sodium citrate on 200m swimming performance

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 2/28/2014

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from 2/6/2013 to 2/28/2014.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 2/28/2014. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at <http://www.brocku.ca/research/policies-and-forms/research-forms>.

In addition, throughout your research, you must report promptly to the REB:

- a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
- c) New information that may adversely affect the safety of the participants or the conduct of the study;
- d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:

Brian Roy, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Appendix 3

Health Canada Letter of Approval



Health
Canada

Santé
Canada

Notice of Authorization

Your file Votre référence

Our file Notre référence

Company Code. 31502

File No. 186236

Submission No. 186236

January 23, 2013

Dr. Nota Klentrou

Panagiota (Nota) Klentrou, Applied Health Sciences, Brock University
500 Glenridge Ave
St Catharines, ON
L2S 3A1

Dear Dr. Klentrou,

Re: CLINICAL TRIAL APPLICATION for Sodium Citrate
Natural Health Products Regulations Section: 67

The Natural Health Products Directorate, Bureau of Clinical Trials and Health Sciences, is pleased to inform you that the information and material provided to support the above Clinical Trial Application, have been assessed and we have no objection to your proposed study. Please consider this as your notice of authorization to sell or import this natural health product for the purposes of this clinical trial in Canada.

I would remind you of the necessity of complying with the *Natural Health Products Regulations, Part 4*, in the sale of this product for clinical testing. In addition, the Regulations (Part 4) impose responsibilities, including commencement notice, record keeping and reaction reporting, on those conducting clinical trials. Please ensure that all systems are compliant in order to meet these responsibilities.

Please note that the mandatory reporting requirements of the NHPR for Serious Adverse Reaction (SARs) that have occurred in Canada will continue to be applied by the Therapeutic Products Directorate (TPD). However, we request that you submit all SAR case reports using the Council for International Organizations of Medical Sciences (CIOMS) I Form. This preferred form can be downloaded and printed at <http://www.cioms.ch/index.php/cioms-form-i>. The SAR report should be faxed to the following number: (613)941-2121.

You are also reminded that all clinical trials should be conducted in compliance with the Health Canada Guidance for Industry: Good Clinical Practice: Consolidated Guideline ICH Topic E6.

Should you have any questions concerning this letter, please contact the submission coordinator at nhpd-cta.dec-dpsn@hc-sc.gc.ca.

Yours sincerely,

Adam Gibson

Canada

Appendix 4

Letter of Invitation

Letter of invitation to participate in a research study:

Effects of acute versus chronic dosing of sodium citrate on 200m swimming performance

Dear swimmer,

I, Colin Russell, am a Masters student with the Faculty of Applied Health Sciences at Brock University, tel: (905) 301-2848, email: cr11af@brocku.ca. I am performing a study on the effects of sodium citrate on swimmers in 200m events. The supervisor of the study is Dr. Nota Klentrou of the Faculty of Applied Health Sciences, email: nklentrou@brocku.ca. Permission to conduct the study has been granted from the Brock University Research Ethics Board.

Male swimmers will randomly perform 4 trials conducted on 4 occasions during racing season (October-April) separated by a length of time equal to or greater than one week. In this study, swimmers will be asked to also complete a specific 200m event of their preferred stroke four times as part of each trial. One trial will be with a compound known as sodium citrate at a high dose, another with sodium citrate at a low dose (chronic dose) but taken over 4 days, three times a day, and the other two with a placebo either at high, single dose or at low dose taken over 4 days. On the race day of each trial the sodium citrate will be taken 100-120 minutes prior to your maximal effort race. Randomization of when the compound or placebo is to be ingested will be in effect.

The swims for the study will be completed in time trial format on a date to be announced. Swimmers will be asked to refrain from activities that require large amounts of energy and could possibly affect performance during the study, and you should not drink alcohol within 12 hours of the testing, or consume caffeine within 6 hours of the testing. The time commitment for each trial will be 2 hours.

We will be asking for information about your date of birth, height, weight, and physical wellbeing as well as best times. A Medical History Questionnaire will be used to screen for general health and medication use that may be a safety concern to the participant. Other information will come from blood work. Blood will be drawn via finger prick. Blood work will be done with an automated lactate analyzer (Arkray Lactate Pro LT-1710 and I-Stat-1) to determine whole blood lactate (Pyne et al., 2000) and other blood variables. With a finger prick there is a small risk of bruising and infection. This blood work will be done pre-ingestion, pre-race and post-race. Lactic acid levels in the blood will be recorded pre-ingestion, pre-race, and post-race.

We hope that the results will contribute to our understanding of sodium citrate and its potential as an ergogenic aid. Sodium citrate is a legal substance that is not banned by the Canadian Centre for Ethics in Sport or the World Anti Doping Agency and could potentially affect the results of performances in swimmers and by extension other athletes. Further, the knowledge gained from the study may assist the coaches and athletes in future competitions.

There may be certain risks associated with participation in the study. The risks and measures to be taken by the researchers to minimize the risks are:

1. Finger bruising and infection will be prevented by adhering to proper technique (disinfecting and cleansing of the fingers before and after the finger prick) for finger pricking. Participants will be provided with a band-aid for their fingers. Bruising will also be minimized by alternating finger to prick.

2. The possible side effects of taking sodium citrate include: stomach ache or cramping, nausea, vomiting, and diarrhea. These risks will be minimized by providing participants with sodium citrate in solution with 500mL of flavoured water to aid in taking a dose that has been previously reported in several research studies in this age range.

Prior to conducting the study, I will obtain written, informed consent from the swimmers. There is no obligation to participate in the study and the swimmer may withdraw from study at any time without affecting the coaching that the swimmer may receive. All information collected during the study will not identify the swimmer and by name.

Thank you for your interest in hearing about the study. If you wish to participate please contact me at the number or email listed below.

Colin Russell, cr11af@brocku.ca, (905) 301-2848

Appendix 5

Parent / Guardian Informed Consent

RESEARCH STUDY INFORMATION AND PARENTAL OR GURADIAN CONSENT

Effects of acute versus chronic dosing of sodium citrate on 200m swimming performance

You child is being asked to take part in a research study. This research is intended to test the effectiveness of sodium citrate as a legal ergogenic supplement for maximal exercise performance. Before agreeing to allow participation in this study, it is important that you read and understand the following explanation of the proposed study procedures. The following information describes the purpose, procedures, benefits, discomforts, risks and precautions associated with this study. It also describes your right to refuse to allow participation or withdrawal from the study at any time. In order to decide whether you wish to allow your child to participate in this research study, you should understand enough about its risks and benefits to be able to make an informed decision. This is known as the informed assent process. Please ask the study doctor (Dr. Nota Klentrou) or study staff to explain any words you don't understand before signing this assent form. Make sure all your questions have been answered to your satisfaction before signing this document.

Purpose

The purpose of this study is to determine the potential performance enhancing effects of sodium citrate on male swimmers 13-17 years of age. Sodium Citrate has been studied in other sports over a broad array of doses, times, and distances with inconclusive results. This study seeks to find any potential effects for a certain distance in a specific sport.

In the course of this study we will monitor the response of various physiological variables involved in keeping your child's blood acid levels balanced, and also their blood lactate levels.

The results of the study will help us understand how the sodium citrate may work to improve athletic performance.

Procedures

Participants will randomly perform 4 trials conducted on 4 occasions during racing season (October-April) separated by a length of time equal to or greater than one

(1) week. More specifically, the swimmers will be asked to also complete a specific 200m event of their preferred stroke four times as part of each trial. One trial will be with a compound known as sodium citrate at a high dose of 0.5g/kg in solution with flavoured water, another with sodium citrate at a low dose (chronic dose) of 0.1 g/kg but taken over 3 days, three times a day and on the fourth day (trial day) one dose at 0.3g/kg also in solution with flavoured water. The other trials will be with a placebo, flavoured water only at the same frequency as the acute or chronic protocol depending on your trial group. On the race day of each trial the sodium citrate will be taken 100-120 minutes prior to your maximal effort race. Warm-up and warm-down procedures will be based solely on each swimmers typical regimen. Randomization of when participants take sodium citrate or placebo will occur.

Physiological data will be collected from you pre-ingestion, pre-race, and post-race. A venous blood sample will be collected after a finger prick and analyzed immediately by an automated lactate analyzer (Arkray Lactate Pro LT-1710 and I-Stat-1) to determine whole blood lactate and other blood variables (see attached list).

Your child will be asked to refrain from abnormal, energy consuming lifestyle choices (staying up late, working out more than normal for a given week, ect.) between the four swims. Participants must refrain from using caffeine and alcohol for 6 and 12 hours respectively before testing. Your child should also maintain a similar diet and supplement use during the study. The time commitment for each trial will be 2 hours.

Risks

There may be certain risks associated with participation in the study. Bruising and infection of the fingers may occur as a result of the finger prick during the lactate test and blood test. Finger bruising and infection will be prevented by adhering to proper technique (disinfecting and cleansing of the fingers before and after the finger prick) for finger pricking. Furthermore, we will alternate fingers when performing a finger prick.

Stomach ache or cramping, nausea, vomiting and/or diarrhea due to sodium citrate ingestion may also occur. Sodium citrate risks will be minimized by providing participants with sodium citrate in solution with 500mL of flavoured water to aid in taking a dose that has been previously reported in several research studies in this age range. After your child stop taking the dose of sodium citrate it should no longer be in their system after 2.5 days under normal circumstances. Dr. Matt J. Greenway will be available in case any problems occur during the study period. All serious adverse events will be immediately reported to Health Canada and any reported minor discomforts will be recorded.

Sodium citrate is a legal substance and is not banned by the Canadian Centre for Ethics in Sport or the World Anti Doping Agency.

Benefits

You will receive no benefit from your participation in this study. Information learned from this study may benefit athletes in the future.

Confidentiality

All information obtained during the study will be held in a password protected server in strict confidence in a locked office at Brock University for a period of 5 years, after which it will be destroyed. Research records will be stored in a confidential manner so as to protect the confidentiality of your information. You will be identified with a study number only. No names or identifying information will be used in any publication or presentations. Health Canada may have access to the records at anytime.

Participation

Your child's participation in this study is voluntary. You can choose not to allow them to participate or you may withdraw them at any time without consequences.

Reimbursements / Compensation

There is no compensation for participation in this study,

Questions

If your child suffers any side effects or other injuries during the study, or if you have any general questions about the study, please see Dr. Matt J. Greenway on site for this study.

If you have any questions about your rights as a parent or guardian of a research participant, please contact the Brock University Research Ethics Officer (905-688-5550 ext 3035; reb@brocku.ca). This study has been reviewed and received ethics clearance through Brock University's Research Ethics Board (file # 12-009) Please keep a copy of this consent form for your records.

Consent

I have had the opportunity to discuss this study and my questions have been answered to my satisfaction. Signing this does not release the investigators from negligence or liability. I consent to take part in the study with the understanding I

may withdraw at any time. I have received a signed copy of this consent form. I voluntarily consent to participate in this study.

Parent or Guardian's Name
(Please Print)

Parent or Guradian's Signature

Date

I confirm that I have explained the nature and purpose of the study to the subject named above. I have answered all questions.

Name of Person
Obtaining Consent

Signature

Date

Appendix 6

Participant Informed Assent

RESEARCH STUDY INFORMATION AND ASSENT

Effects of acute versus chronic dosing of sodium citrate on 200m swimming performance

You are being asked to take part in a research study. This research is intended to test the effectiveness of sodium citrate as a legal ergogenic supplement for maximal exercise performance. Before agreeing to participate in this study, it is important that you read and understand the following explanation of the proposed study procedures. The following information describes the purpose, procedures, benefits, discomforts, risks and precautions associated with this study. It also describes your right to refuse to participate or withdraw from the study at any time. In order to decide whether you wish to participate in this research study, you should understand enough about its risks and benefits to be able to make an informed decision. This is known as the informed consent process. Please ask the study doctor (Dr. Nota Klentrou) or study staff to explain any words you don't understand before signing this consent form. Make sure all your questions have been answered to your satisfaction before signing this document.

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The purpose of this study is to determine the potential performance enhancing effects of sodium citrate on male swimmers 13-17 years of age. Sodium Citrate has been studied in other sports over a broad array of doses, times, and distances with inconclusive results. This study seeks to find any potential effects for a certain distance in a specific sport.

In the course of this study we will monitor the response of various physiological variables involved in keeping your blood acid levels balanced, and also your blood lactate levels.

The results of the study will help us understand how the sodium citrate may work to improve athletic performance.

Procedures

Participants will randomly perform 4 trials conducted on 4 occasions during racing season (October-April) separated by a length of time equal to or greater than one (1) week. More specifically, the swimmers will be asked to also complete a specific

200m event of their preferred stroke four times as part of each trial. One trial will be with a compound known as sodium citrate at a high dose of 0.5g/kg in solution with flavoured water, another with sodium citrate at a low dose (chronic dose) of 0.1 g/kg but taken over 3 days, three times a day and on the fourth day (trial day) one dose at 0.3g/kg also in solution with flavoured water. The other trials will be with a placebo, flavoured water only at the same frequency as the acute or chronic protocol depending on your trial group. On the race day of each trial the sodium citrate will be taken 100-120 minutes prior to your maximal effort race. Warm-up and warm-down procedures will be based solely on each swimmers typical regimen. Randomization of when participants take sodium citrate or placebo will occur.

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You will be asked to refrain from abnormal, energy consuming lifestyle choices (staying up late, working out more than normal for a given week, ect.) between the two swims. Participants must refrain from using caffeine and alcohol for 6 and 12 hours respectively before testing. You should also maintain a similar diet and supplement use during the study. The time commitment for each trial will be 2 hours.

Risks

There may be certain risks associated with participation in the study. Bruising and infection of the fingers may occur as a result of the finger prick during the lactate test and blood test. Finger bruising and infection will be prevented by adhering to proper technique (disinfecting and cleansing of the fingers before and after the finger prick) for finger pricking. Furthermore, we will alternate fingers when performing a finger prick.

Stomach ache or cramping, nausea, vomiting and/or diarrhea due to sodium citrate ingestion may also occur. Sodium citrate risks will be minimized by providing participants with sodium citrate in solution of 500mL of flavoured water to aid in taking a dose that has been previously reported in several research studies in this age range. After you stop taking the dose of sodium citrate it should no longer be in your system after 2.5 days under normal circumstances. Dr. Matt J. Greenway will be available in case any problems occur during the study period. All serious adverse events will be immediately reported to Health Canada and any reported minor discomforts will be recorded.

Sodium citrate is a legal substance and is not banned by the Canadian Centre for Ethics in Sport or the World Anti Doping Agency.

Benefits

You will receive no benefit from your participation in this study. Information learned from this study may benefit athletes in the future.

Confidentiality

All information obtained during the study will be held in a password protected server in strict confidence in a locked office at Brock University for a period of 25 years, after which it will be destroyed. Research records will be stored in a confidential manner so as to protect the confidentiality of your information. You will be identified with a study number only. No names or identifying information will be used in any publication or presentations. Health Canada may have access to the study records at anytime.

Participation

Your participation in this study is voluntary. You can choose not to participate or you may withdraw at any time without consequences.

Reimbursements / Compensation

There is no compensation for participation in this study,

Questions

If you suffer any side effects or other injuries during the study, or if you have any general questions about the study, please see Dr. Matt J. Greenway on site for the study.

If you have any questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905-688-5550 ext 3035; reb@brocku.ca). This study has been reviewed and received ethics clearance through Brock University's Research Ethics Board (file # 12-009). Please keep a copy of this consent form for your records.

Consent

I have had the opportunity to discuss this study and my questions have been answered to my satisfaction. Signing this does not release the investigators from negligence or liability. I consent to take part in the study with the understanding I may withdraw at any time. I have received a signed copy of this consent form. I voluntarily consent to participate in this study.

Study Participant's Name
(Please Print)

Study Participant's Signature

Date

I confirm that I have explained the nature and purpose of the study to the subject named above. I have answered all questions.

Name of Person
Obtaining Consent

Signature

Date

Appendix 7

Raw Data

Participant	Trial Order	Age (yrs)		Doses (g)						Lactate (mmol/L)		
			BM (kg)	0.1	0.3	0.5	Stroke	Time (s)	RPE	Lac 1	Lac 2	Lac 3
1	1	15	69.5	7.0	20.9	34.8	Backstroke	145.5	17	0.94	1.6	10.7
	2							145.7	17	1	2.1	10
	3							145.7	17	1	0.9	12.2
	4							145.9	18	1.14	0.71	8.6
2	1	16	74.1	7.4	22.2	37.1	I.M.	154.5	17	1.4	0.9	7.4
	2							154.6	12	1.1	4.3	6.4
	4							153.8	16	1.8	1.8	7.3
	3							158.6	18	0.8	1.6	4.9
3	2	16	83.6	8.4	25.1	41.8	Freestyle	125.66	18	2.2	1.9	13.6
	1							126.5	18	1.4	1.6	10.3
	3							126.6	16	1	1.8	12.3
	4							124.9	19	1.1	1.1	13.6
4	1	16	64.2	6.4	19.3	32.1	Freestyle	130.5	17	0.62	1.1	6.4
	3							127.9	17	0.7	0.7	8.2
	2							128	16	0.7	0.8	8.4
	4							128.4	18	1.83	0.7	8.6
5	2	17	77.3	7.7	23.2	38.7	Freestyle	129.5	18	2.3	1.1	15.7
	3							129.6	15	0.9	1.1	9.8
	1							131.2	16	1.16	0.9	9.9
	4							130.4	18	1	0.8	10.8
6	1	14	56.5	5.7	17.0	28.3	Freestyle	135.5	18	1.18	1.2	5.3
	2							136.2	16	0.9	1.9	4.3
	4							133.3	16	0.7	0.8	5.7
	3							131	17	1.4	1.7	5.9
7	2	14	49.1	4.9	14.7	24.6	Breaststroke	168.5	17	1.1	1.2	5.6
	3							170.4	16	0.9	0.8	5.7
	1							161.6	13	1.4	1.8	8.8
	4							164.6	13	1.4	1.3	7.9
8	2	15	62.5	6.3	18.8	31.3	Freestyle	126.5	15	1.2	1.4	15
	4							126.5	17	1	1.6	10.1
	1							127.8	14	1.4	1.2	8.6
	3							127.7	16	0.84	1.1	9.9
9	2	13	47.7	4.8	14.3	23.9	Freestyle	159.5	17	1.2	1.2	7.2
	1							155.8	15	1.2	1.3	7
	3							155.1	16	1.8	1.1	6.9
	4							156.5	17	1.7	0.9	15.1
10	1	13	50.6	5.1	15.3	25.5	Breaststroke	166.0	19	1.2	0.9	12.1
	4							170.7	19	0.9	1.3	11.7
	3							164	18	1.1	1.9	13.8
	2							165.3	15	1.7	1	12.2
	1=PLC-A	3=CHR										

	2=ACU	4=PLC-C										
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Trial	BE1 (mmol/L)	BE2	BE3	BICARB1 (mmol/L)	BICARB2	BICARB3	PCO2_1 (mmHg)	PCO2_2	PCO2_3
1	1	-2	-13	24.6	24	14.3	39.2	37.6	33
1	-2	-2		22.5	22.8		36.7	36.2	
1	2	-1		26.6	25		40	39.8	
1	0	-1		26.3	22.5		43.2	34.1	
1	0	0		24.6	23		39.1	35.2	
1	1			25			38.9		
1	2			26.5			42		
2	1	8	-3	25.2	31.3	22.5	39.3	40.4	40.6
2	-1	3	-11	23.6	27	16	35.8	39.3	34.4
2	-2	8	-3	23.1	31.6	21.9	36.8	41.9	37.5
2	-1	9	-12	23.4	32.1	14.7	35.5	41.8	29.8
2	0	6		24.5	28.7		38.5	34.9	
3	1	6	-7	24.8	29.3	19	36.2	39.4	34.4
3	1	0	-10	25.6	24.3	16.3	37.7	36.8	34.3
3	2	7	-9	27	29.6	17.1	42.5	35	35.3
3	0	5	-15	24.3	29.1	12.8	37.8	41.8	31.3
3	2	6	-3	26.1	29	22.4	40.2	37.4	39.1
3	1	6		24.4	29.9		34.4	40.2	
4	1	1	-12	25.4	24.9	15.4	38.2	36.9	34.3
4	1	-2	-19	25.6	22.8	10.3	38.5	35.6	29.8
4	1	-2	-17	24.6	23	11.6	35.6	37.1	30.8
4	2	-1	-7	26	23.6	18.6	39.3	36.4	35.4
4	1	1	-10	25.6	25.1	16.6	41.8	38.9	36.7
4	0	1	-11	24.7	25.2	15.9	38.9	38	35.9
4	1		-14	25.2		14.2	39.7		35.5
4			-5			21			38.1